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CONTRIBUTION TO THE KNOWLEDGE OF THE COLLOID CHEMISTRY OF GLUTEN. IV

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Lyotropy

In a preceding paper (1930) the influence of acid on the viscosity of gliadin sols was investigated. It was proven that the separation phenomenon was a gradual one. This paper will deal with the influence of electrolytes on the viscosity of gliadin sols in aqueous as well as in acetone medium. These investigations will also give certain data relative to the viscosity effects caused by the lyotropy and the charge of the ions. The results obtained confirm the graduality of the separation phenomena and the increase in stability in definite acetone-water mixtures.

If one considers the Einstein-Smoluchowski formula in its most simple form $\frac{\eta s - \eta o}{\eta o} = K\phi\ (1 + \delta\zeta_2)$ it will be observed that a decrease in the first term can be accomplished either as the result of a decrease of $\delta\zeta_2$ or as the result of a decrease of ϕ. By the addition of equivalent concentrations of electrolytes of the same valency a difference in the decrease in viscosity of the protein solutions can be observed. This decrease in viscosity can be explained as, (1) being brought about by a decrease of charge (quasi-viscous effect), the same for all electrolytes of the same valency; and (2) a decrease in viscosity varying for electrolytes of the same valency (lyotropic influence).

As long as a colloid is charged positively, the electrolyte will direct its negative ions towards the particle. So in this case the lyotropic influence will be exerted mainly by the negative ions. The lyotropic series for the univalent ions runs as follows: Fl, Cl, Br, NO_3, I, CNS, where CNS has the strongest dehydrating influence.

In a paper published by us (1928) in this journal, it was pointed out that, in considering the lyotropic influence, one can not compare the bivalent SO_4-ion with the univalent Cl, Br, NO_3, I, and CNS ions as long as the colloid particles are in a charged condition. In fact, as

long as the charge is one of the stability factors of the particles, the discharging effect of the ions will out-balance the lyotropic influence. Only when the particles are practically discharged will it be possible to compare the lyotropic effects of the ions of different valency, whereas the lyotropic effect of ions of the same valency can be compared during the discharging process. In the last mentioned case the differences in decrease in viscosity will only be caused by the different lyotropic influence, the fall in viscosity caused by the charge being the same for equivalent concentrations of these electrolytes. Therefore, the SO_4-ion does not belong to the lyotropic series of the univalent ions as long as the colloid particles are charged. However, by salting-out processes, when the protein is practically discharged, the SO_4-ion can be put into the lyotropic series.

Experimental

Aqueous Medium

By the addition of electrolytes to an acid gliadin sol it was found, in small concentrations, that the SO_4-ion did not take its place amongst the monovalent ions in the lyotropic series. The aim of the following experiments was to ascertain whether this effect was independent of the state of charge of the particles in a sol. In other words, if, at different H-ion concentrations, the effect of charge of the SO_4-ion would always out-weigh the lyotropic influence.

For this purpose different gliadin sols were made at various points in the positive pH region, i.e., close to the iso-electric point, on both sides of the maximum of hydration and at the maximum. The decrease in viscosity caused by the addition of different equivalent, concentrations of K_2SO_4 and KI was determined. The sols were made by shaking 1 gram of air-dry gliadin with 110 cc. of liquid containing different quantities of acid (HCl). The results are given in Table I. From these data it is evident, (1) that at all of the H-ion concentrations investigated that the K_2SO_4 curve at first runs below the KI curve, but with increasing electrolyte concentration a point of intersection is reached; and (2), that this point of intersection shifts with the initial H-ion concentration of the sols. The higher the charge of the sol, the higher will be the electrolyte concentration at which the K_2SO_4 and the KI curves intersect. From this it must follow that at small electrolyte concentrations the discharging effect of K_2SO_4 predominates, but when the charge of the sol is diminished through the discharging of the electrolytes, the curves intersect and the lyotropic influence predominates. For the same reason the point of intersection will shift to smaller electrolyte concentrations when the initial sol has passed its maximal charge. In other words, the electrolyte concen-

tration at which the lyotropic influence begins to predominate is dependent upon the initial charge of the sol.

TABLE I

INFLUENCE OF VARYING CONCENTRATIONS OF KI AND K_2SO_4 ON THE VISCOSITY OF ACID-GLIADIN SOLS OF DIFFERENT H-ION CONCENTRATIONS

	109.5 cc. H_2O, 0.5 cc. N/10 HCl						
Mil. eq. per L.	0	1	2.5	5	10		
KI Series	1.032	1.022	1.019	1.015	1.010		η
	op.	op.	cl.	cl.	cl.		appearance
K_2SO_4 Series	1.032	1.020	1.017	1.016	1.012		η
	op.	sl. cl.	cl.	cl.	cl.		appearance
	107.5 cc. H_2O, 2.5 cc. N/10 HCl						
Mil. eq. per L.	0	1	2.5	5	10	15	
KI Series	1.122	1.086	1.065	1.045	1.021	1.011	η
	—	—	—	sl. cl.	cl.	cl.	appearance
K_2SO_4 Series	1.122	1.069	1.050	1.040	1.031	1.025	η
	—	—	—	sl. cl.	cl.	cl.	appearance
	105 cc. H_2O, 5 cc. N/10 HCl						
Mil. eq. per L.	0	1	2.5	5	10		
KI Series	1.127	1.100	1.078	1.056	1.029		η
	—	—	—	—	sl. cl.		appearance
K_2SO_4 Series	1.127	1.081	1.063	1.050	1.038		η
	—	—	—	sl. cl.	cl.		appearance
	100 cc. H_2O, 10 cc. N/10 HCl						
Mil. eq. per L.	0	1	2.5	5	10		
KI Series	1.107	1.091	1.076	1.058	1.034		η
	—	—	sl. op.	sl. op.	—		appearance
K_2SC_4 Series	1.017	1.078	1.065	1.055	1.044		η
	—	—	sl. op.	sl. op.	—		appearance

ACETONE MEDIUM

In investigations previously reported (1930a), the influence of varying acetone concentrations on the viscosity of an acid gliadin sol was studied. It was pointed out that in acetone of 44 per cent concentration by volume, the relative viscosity of these solutions passes through a maximum. This phenomenon exists in separated as well as in clear solutions. At this maximum, the acetone exercises its maximal stabilizing effect on the protein particle. It was assumed that this could be explained by considering the particle as surrounded by an acetone-hydrate layer. In concentrations of more than 44 per cent, the gliadin gradually lost its protecting layer and was only stable on its charge; the emulsoid had passed into a suspensoid. This part of the study deals with the influence of acetone on gliadin solutions separated with different electrolytes.

On adding acetone to aqueous sols separated with electrolytes, the solutions became watery clear. In concentrations of more than 44

per cent acetone a second turbidity appears, and at a sufficiently high concentration of acetone flocculation results, on account of the discharging of the practically desolvated particle.

TABLE II

INFLUENCE OF VARYING ACETONE CONCENTRATIONS ON THE RELATIVE VISCOSITY OF GLIADIN SOLS WITH 4 MILLIMOLES KCl AND KI

Acetone—cc.	0	3	6	9	12	15	18	20
				KCl Series				
ηAc	....	1.233	1.443	1.579	1.600	1.479	1.221	0.987
$\eta s+Ac$	1.047	1.308	1.547	1.705	1.730	1.590	1.286	1.005
$\frac{\eta s+Ac}{\eta Ac}$	1.047	1.061	1.072	1.079	1.081	1.075	1.053	1.013
Appearance	sl. cl.[1]	sl. op.[2]						cl. flocc.[3]
				KI Series				
$\eta s+Ac$	1.029	1.298	1.540	1.702	1.729	1.591	1.292	1.016
$\frac{\eta s+Ac}{\eta Ac}$	....	1.053	1.067	1.078	1.081	1.075	1.058	1.024
Appearance	cl.[4]	sl. op.[2]						op.[5]

[1] sl. cl. = slightly cloudy.
[2] sl. op. = slightly opalescent.
[3] cl. flocc. = cloudly flocculated.
[4] cl. = cloudy.
[5] op. = opalescent.

In Table II is listed a series of viscosity measurements for KCl and KI. For this study 2.5 cc. of an acid gliadin sol (obtained by shaking 3 grams of gliadin with 104 cc. of H_2O and 6 cc. of N/10 HCl) were put in volumetric flasks of 25 cc. capacity, containing varying quantities of acetone and 4 millimoles of KCl or KI. The solution was made up to the mark with distilled water at 25° C. It appears that, in acetone concentration of 44 per cent, the viscosities of the KCl and the KI sols run together, while at higher concentrations a second spreading occurs. Here, however, the lyotropic series is reversed (see Figure 1).

The aim of our next experiments was to determine whether this coincidence of the relative viscosities of sols with 4 millimoles of KCl and KI in acetone of about 44 per cent concentration was the case for all concentrations of these electrolytes. Table III gives the results of these measurements. If these data are examined, the following facts are observable: (1) The relative viscosities of sols with equivalent concentrations of KCl and KI are the same (within experimental error) in an acetone medium of about 44 per cent. (2) By increasing the electrolyte concentration in the sols a gradual decrease in the relative viscosity takes place, until the sols are practically discharged. Here the viscosity curve runs in a horizontal direction.

It was further thought desirable to investigate whether this inter-

section of the relative viscosities was dependent upon the nature of the electrolyte and only held good for KCl and KI, or whether the viscosity curves for all the monovalent electrolytes intersected one another at this maximum. This would make it probable that only

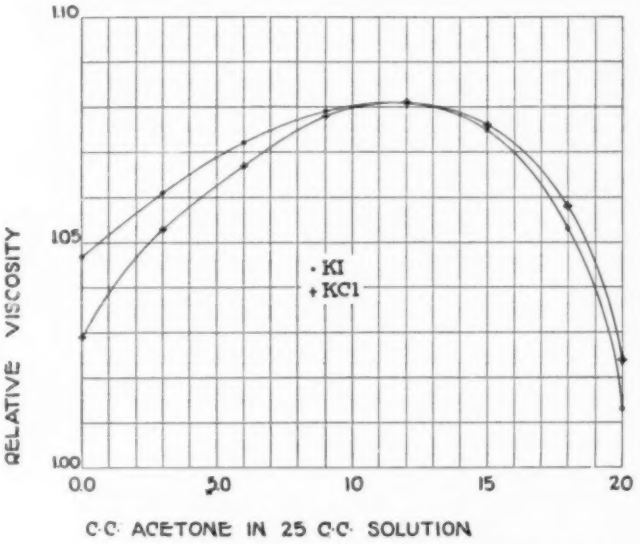

Fig. 1. Influence of varying acetone concentration on the relative viscosity of gliadin sols containing 4 millimoles KCl and KI. See Table II.

the state of the protein particle in this medium was of importance, and not the structure of the ion. Table IV lists a series of measurements for different monovalent ions. From these data it can be concluded that, (1) with increasing acetone concentrations the relative viscosities of the sols with equivalent quantities of different monovalent ions increase and approach each other, (2) that within experimental error, the viscosities in 44 per cent acetone are the same, (3) that in acetone of more than 44 per cent concentration a second spreading of the curves occurs, and (4) that, until a concentration of 44 per cent acetone has been reached, the lyotropic series has the same order as in aqueous medium, this order is reversed in concentration of more than 44 per cent.

These measurements were repeated in alcohol media, with analogous results.

Concerning the influence of the protein concentration on these phenomena it can be said that at the higher concentrations small differences were found. To develop a discussion of these measurements and to explain them would lead us afar from the objective of this paper.

TABLE III

INFLUENCE OF VARYING CONCENTRATIONS OF KI AND KCl ON THE RELATIVE VISCOSITY OF AN ACID 44 PER CENT ACETONE GLIADIN SOL

Milli equivalents of electrolyte	0	2	4	6	8	10	12	20	28	40	80
$\eta_s + Ac$ KCl	1.805	1.737	1.722	1.716	1.713	1.703	1.702	1.697	1.692	1.695	1.694
$\eta Ac + w$ KCl	1.602	—	—	—	—	—	1.605	1.600	1.597	1.601	1.602
$\eta_s + Ac$ KI	1.805	1.742	1.722	1.714	1.711	1.702	1.698	1.694	1.688	1.685	1.677
$\eta Ac + w$ KI	1.602	—	—	—	—	—	1.600	1.597	1.593	1.594	1.587
$\frac{\eta_s + Ac}{\eta Ac}$ KI	1.127	1.088	1.075	1.070	1.069	1.063	1.062	1.061	1.060	1.057	1.057
$\frac{\eta_s + Ac}{\eta Ac}$ KCl	1.127	1.086	1.075	1.071	1.070	1.064	1.061	1.060	1.060	1.059	1.058

TABLE IV

INFLUENCE OF VARYING ACETONE CONCENTRATIONS ON THE RELATIVE VISCOSITY OF ACID GLIADIN SOLS WITH 6 MILLIMOLES OF DIFFERENT UNIVALENT ELECTROLYTES

Electrolyte used	cc. Acetone							
	0	3	6	10	12	15	18	
Without electrolyte	—	1.359	1.616	—	1.823	—	1.351	$\eta s + Ac$
	1.090	1.102	1.120	—	1.140	—	1.106	$\frac{\eta s + Ac}{\eta Ac}$
CH_3SO_3Na	—	1.304	1.539	—	1.719	—	1.282	$\eta s + Ac$
	1.040	1.057	1.067	—	1.074	—	1.050	$\frac{\eta s + Ac}{\eta Ac}$
KCl	—	1.299	1.538	1.716	1.719	1.582	1.274	$\eta s + Ac$
	1.036	1.053	1.066	1.073	1.074	1.069	1.044	$\frac{\eta s + Ac}{\eta Ac}$
KBr	—	1.293	1.535	1.716	1.717	1.582	—	$\eta s + Ac$
	1.032	1.049	1.064	1.073	1.073	1.069	—	$\frac{\eta s + Ac}{\eta Ac}$
KNO_3	—	1.293	1.534	1.718	1.717	1.583	1.280	$\eta s + Ac$
	1.029	1.049	1.063	1.074	1.073	1.070	1.049	$\frac{\eta s + Ac}{\eta Ac}$
KI	—	—	—	—	1.718	—	1.282	$\eta s + Ac$
	1.018	—	—	—	1.073	—	1.050	$\frac{\eta s + Ac}{\eta Ac}$
KCNS	—	1.286	1.528	1.716	1.719	1.584	1.289	$\eta s + Ac$
	1.015	1.043	1.059	1.073	1.074	1.071	1.056	$\frac{\eta s + Ac}{\eta Ac}$
	—	.09108	.15932	.20404	.20404	.16994	.08664	$\log \eta A$

POLYVALENT IONS

To study the influence of the bi-valent ions the same method as for the monovalent ions was followed. Here, too, the change in the relative viscosity of gliadin sols with a constant quantity of K_2SO_4, but with varying acetone concentrations, was studied.

The shape of the curve was similar to that of the sols with monovalent ions. There is a maximum at a 44 per cent acetone concentration and in higher concentrations flocculation results. On comparing the curves for equivalent concentrations of KCl and K_2SO_4 it appears that the maximum for the sol containing sulfate was lower than that for the sol with KCl. This was to be expected as the bi-valent sulfate ion has the stronger discharging influence.

Having ascertained the fact that the shape of the curve is the same for ions of mon- and bi-valency, the influence of changing concentration of ions of different valency in 44 per cent acetone concentration was next studied. Table V records the data.

TABLE V

INFLUENCE OF VARYING CONCENTRATIONS OF DIFFERENT VALENT ELECTROLYTES ON THE RELATIVE VISCOSITY OF AN ACID 44 PER CENT ACETONIC-GLIADIN WITH MAXIMAL VISCOSITY

Electrolyte used	Milli equivalent of electrolytes													
	0	½	1	2	4	6	8	10	12	16	20	30	36	
KCl	1.824	—	1.758	1.740	1.727	1.720	1.718	1.711	—	—	1.702	1.701	—	$\eta s + Ac$
	1.140	—	1.099	1.088	1.080	1.075	1.074	1.069	—	—	1.061	1.061	—	$\frac{\eta s + Ac}{\eta Ac + \text{electrolyte}}$
K_2SO_4	1.824	—	1.711	1.699	1.697	1.692	1.691	1.686	1.688	—	1.689	1.695	1.697	$\eta s + Ac$
	1.140	—	1.070	1.062	1.060	1.057	1.057	1.055	1.053	—	1.053	1.053	1.051	$\frac{\eta s + Ac}{\eta Ac + \text{electrolyte}}$
K_4FeCy_6[6]	1.824	1.694	1.669	1.677	1.678	1.680	1.680	1.681	1.684	1.690	1.694	1.701	1.703	$\eta s + Ac$
	1.140	1.059	1.043	1.048	1.049	1.050	1.050	1.051	1.051	1.052	1.054	1.053	1.055	$\frac{\eta s + Ac}{\eta Ac + \text{electrolyte}}$
			cl.[1]	v.cl.[2]	sl.cl.[3]									

[1] cl. = cloudy.
[2] v.cl. = very cloudy.
[3] sl.cl. = slightly cloudy.

Whereas the sols containing varying quantities of KCl and K_2SO_4 were always perfectly clear, this was not the case with K_4FeCy_6. The first traces of K_4FeCy_6 caused a sharp drop in the viscosity. At about 1 to 4 milli equivalents the solutions were more or less cloudy. On addition of more K_4FeCy_6 the solutions again became clear and there was a small rise in the viscosity. When these data are graphed (Figure 2) the following facts can be observed: (1) by the discharging of acid

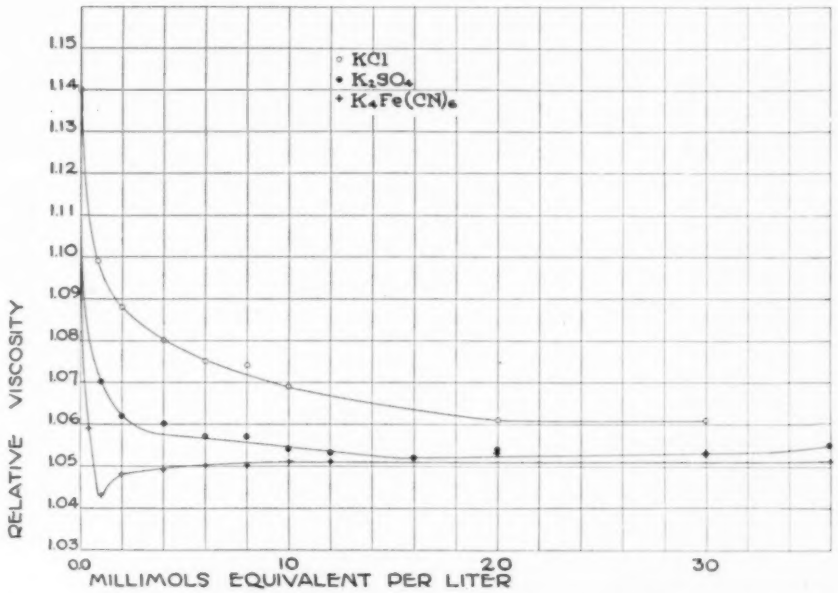

Fig. 2. Influence of varying concentrations of different valent electrolytes on the relative viscosity of an acid 44 per cent acetone gliadin sol. See Table V.

gliadin sols containing 44 per cent acetone, there is a distinct spreading of the relative viscosity curves for ions of different valency, and (2) the viscosities of these discharged sols do not come to the same level.

Influence of Changing H-Ion Concentration on 44 Per cent Acetone Gliadin Sols Containing Electrolyte

We next investigated the effect of changing pH on the relative viscosity of a sol containing varying quantities of KCl. The following method was used for these experiments: "1.3636 grams of gliadin was shaken with 50 cc. of a mixture of acetone and water in a thermostat at 25° C. This acetone water mixture was made by diluting 200 cc. of acetone with distilled water to 500 cc. in a volumetric flask at 25° C. The protein solution thus obtained was not filtered in order to avoid evaporation. 2.5 cc. of this solution were pipetted into a volumetric

flask with 11 cc. acetone and the necessary quantities of acid and electrolyte, and were made up to 25 cc. at 25° C." In this way the protein as well as the acetone concentration were practically the same as those used in our former experiments.

TABLE VI

INFLUENCE OF CHANGING ACID CONCENTRATION ON THE RELATIVE VISCOSITY OF A GLIADIN SOL WITH VARYING KCl CONCENTRATION IN ACETONE 44 PER CENT BY WEIGHT

Mil. eq. HCl per L.	Milli equivalents of KCl per liter					
	0	3	6	8	10	14
0	1.689 1.056	1.687 1.055	1.688 1.056	1.688 1.055	1.692 1.058	1.689 1.056
0.2	1.730 1.081	1.699 1.062	1.697 1.061	1.692 1.058	1.692 1.058	1.692 1.058
0.4	1.775 1.110	1.713 1.071	1.705 1.066	1.705 1.065	1.704 1.065	1.701 1.063
0.6	1.812 1.113	1.724 1.077	1.714 1.072	1.712 1.070	1.709 1.068	1.703 1.065
0.8	1.826 1.141	1.727 1.080	1.718 1.074	1.715 1.072	1.710 1.069	1.710 1.069
1.2	1.808 1.130	1.740 1.088	1.726 1.079	1.723 1.077	1.718 1.074	1.716 1.073
1.6	1.794 1.122	1.735 1.085	1.728 1.080	— —	1.717 1.074	1.715 1.072
4.0	1.748 1.092	1.727 1.080	1.718 1.074	— —	— —	1.712 1.070

Table VI serves best to illustrate the influence of changing acid concentration on the relative viscosity of a gliadin sol. From this table it is evident, (1) that with increasing charge of the sol the discharging effect of KCl is more prominent, and (2) that the level of the relative viscosity obtained by discharging differently charged sols with 10 to 14 milli equivalents of KCl rises with increasing charge of the initial sol. This rise in level does not stop at the pH at which the sol, without electrolyte, has its maximal viscosity, but is reached at a slightly higher H-ion concentration. In other words, the relative viscosity maxima of a sol with and without electrolyte are found at different H-ion concentrations.

These investigations were repeated with polyvalent ions, but here the phenomena were much more complicated on account of the reversal of the charge of the sol.

Discussion of Results

The following principal facts can be deduced from the foregoing experimental work on dilute protein solutions: (*a*) By increasing the acetone or alcohol concentration in a sol containing equivalent concentrations of electrolytes of the same valency the differences in the relative viscosity as found in aqueous medium, decrease. See Table II. (*b*) In definite acetone or alcohol concentrations this difference disappears (within the experimental error) to reappear in higher concentrations of these media. See Table IV. (*c*) The value of the relative viscosity in the maximum of the acetone (likewise alcohol) relative viscosity curve is dependent upon the quantity of electrolyte and on its valency. See Table VI.

In explaining these effects, for the sake of briefness, only the phenomena in alcohol media will be discussed. However, the same explanation is applicable to acetone media. The phenomena mentioned above in items *a*, *b*, and *c* can be considered as a change in solvation through a change in medium. Although theoretically it is possible that this change in solvation takes place with the ions as well as with the colloid particle, several reasons, not mentioned here, can be given that make it nearly certain that the greater part of the effects is caused by the change in the solvation layer of the particle.

As we pointed out in a former publication (1930a) the maximum in the relative viscosity curve with changing alcohol concentration can be explained with the forming of an alcohol-hydrate layer on the particle. We assumed that with increasing alcohol the hydrate in the medium, as well as in the solvation layer, is increased. At the maximum, the layer consists chiefly of hydrate molecules. After this point the hydrate in the layer and in the dispersion medium will decrease, but as the gliadin is alcoholophobic, at the same time, the layer will gradually become relatively richer in water than the surrounding medium. Determinations were made on the concentration of alcohol in the solvation layer, and in the dispersion medium, and have proven the correctness of this theory.

In very high alcohol concentration the particle will be practically dehydrated. It can be said that the left-hand part of the viscosity curve represents a system of increasing solvation with increasing alcohol concentration, whereas the right-hand part of the curve gives a system of decreasing solvation, with increasing alcohol concentration. By the addition of equivalent quantities of electrolytes of the same valency to an aqueous gliadin sol, solutions of different viscosities are obtained, i.e., sols of different hydration.

In the first instance, it may be assumed that the discharging effect of equivalent concentrations of ions of the same valency is the same.

The differences found in viscosity for these concentrations indicate that the electrolytes are differently adsorbed by the gliadin particle. This difference in adsorption has been pointed out more than once in the literature of colloid chemistry. The quantity of water expelled from the protecting layer by these different adsorptions can not be the same, therefore these effects must cause a difference in viscosity of these sols.

It stands to reason that this kind of adsorption must be of a different nature than that cuasing the discharging of the particles, otherwise the state of charge of the particles in solutions containing equivalent quantities of electrolytes of the same valency could not be the same. It is necessary, therefore, to distinguish between two kinds of adsorption, the one causing the discharge of the particle through the adsorption of ions of opposite charge, the other being an adsorption of both positive and negative ions of molecules, which causes an alteration of the hydration of the sol without influencing the boundary potential. An indication of the existence of this second kind of adsorption will be found in the investigations of Weiser (1925), Dhar (1924), Huyzing (1928), Schilow (1920), and other investigators. At the same time, in the analogous shape of the curves obtained by adding alcohol to gliadin sols containing surface-active material or electrolytes, we see an indication of this kind of adsorption.

Before discussing the influence of electrolyte on an alcohol sol, it is necessary to consider for a moment the condition of a sol particle in aqueous medium. In an acid gliadin sol the particles are charged positively by the adsorption of H-ions on the surface. The following question now arises: Is the whole surface of the particle entirely or only partly covered by these ions? A rough calculation will give an insight into this question. Suppose the particle to be spherical and the specific gravity of the gliadin to be 1. If the radius of the H-ion is known, it is possible to calculate the area that can be covered by all the H-ions of the acid in solution. For the diameter of the ion we have taken the diameter of the atom. At the same time a simple calculation will give the diameter of the globules, wherein a known quantity of gliadin must be divided so that the total surface of these globules will be the same as the area that can be covered entirely by the H-ions. In the case of the gliadin solution used in our experiments (1.5 gram gliadin + 4 cc. N/10 HCl), the diameter of the particles must be about 4 μ, if the whole surface is to be covered. Now the particles in such a sol are amicroscopic, that is to say, they have a diameter of less than 0.1 μ. For the same quantity of gliadin the total surface of the particles with this diameter will be larger than of particles with a diameter of 4 μ. Consequently, it is impossible for the H-ions of the

acid to cover this surface completely. Besides, only a small part of the H-ions in the medium is adsorbed by the protein, as appears from the slight differences in H-ion concentration of the acid solutions with and without gliadin. It can be concluded then, that only a small part of the surface of the gliadin particles is taken up by the H-ions. These adsorbed H-ions are centres of hydration, between these centres are large areas unprotected by this hydration, caused by charge. Nevertheless, it must be assumed that these uncharged parts of the surface are protected by a hydration layer. This water is attracted as a result of the polar character of the protein molecules. On the addition of electrolytes to such a sol part of the electrolyte is adsorbed by these uncharged parts and the water is expelled from the surface.

Let us now discuss the influence of alcohol on gliadin sols containing electrolytes. In our opinion the forming of an hydrate layer cannot give a full explanation of the phenomena mentioned in items *a* and *b* on page 449. We assume, that coupled with the increase of alcohol hydrate molecules on the surface of the particles, caused by the increase of the alcohol concentration in the medium, that the electrolyte molecularly adsorbed will be expelled gradually from the surface. In the maximum, the particles will be bare of electrolytes or they will be present in the same quantity. Since, in aqueous solutions of equimolar concentrations, the quantity of KI adsorbed on the surface of the protein is larger than the quantity of KCl, by the addition of alcohol a larger quantity of KI than of KCl will be thrown off the surface. Therefore, the rise in relative viscosity will be larger for the KI sol than for the KCl sol, and in consequence, the relative viscosity curve will be steeper for the KI sol. In the deportment of a sol containing electrolyte in varying alcohol media we see an analogy of the conduct of a sol containing surface active material in varying alcohol media. In the last mentioned case, it is assumed that the increase of the relative viscosity of a sol containing, for instance, small quantities of resorcinol, is caused by the disappearance of the adsorbed substance from the surface of the particle. Up until now we have not been able to test our supposition, that, in the case of sols containing electrolyte, this will be expelled from the particle by the addition of alcohol. Ultrafiltration of these alcoholic sols was impossible. On the other hand, all experiments indicate that there still exists at the alcohol or acetone concentration, at which maximum viscosity occurs, a certain sensibility of the sol for electrolyte. However, the effects are the same for equivalent concentrations of electrolytes of the same valency, but different for electrolytes of different valency. The higher the valency of the negative ion, the more pronounced will be the influence of the electrolyte.

According to the before mentioned explanation, the protecting layer in the maximum is of such a formation that the effects of the molecularly adsorbed electrolytes have disappeared, though there still exists in this medium an essentially different kind of adsorption. Because of the differences in the relative viscosity found for sols containing equivalent concentrations of electrolytes with anions of different valency, this adsorption must be an ion adsorption, which only influences the boundary potential. Thus, in Tables III, V, and VI, the decrease in relative viscosity in the maximum is only caused by discharging effects. So this represents the purely electro-viscous effect, while this effect in aqueous medium is always coupled with lyotropic influences.

Let us now consider the right hand part of the relative viscosity-alcohol curve. In the first instance, the same phenomena will occur as with a sol without electrolyte, i.e., a decrease of the hydrate in the solvation layer and a relative increase of water in the layer. But coupled herewith there will be, by increasing alcohol concentration, a decrease in solubility of the electrolyte, which is larger for KCl than for KI. Data with respect to this are to be found in the literature. Because the solvation layer is relatively richer in water than the dispersion medium, the KCl will be driven to the water-rich phase and adsorb on the surface of the particles. KI will do the same, but to a less extent. In these media we will thus get a reverse of the lyotropic series. An analogous explanation can be given for the other monovalent ions.

A brief discussion of the viscosimetric measurement in 44 per cent acetone medium with sols containing varying quantities of mono- and poly-valent electrolytes, especially those with K_4FeCy_6 (Table V), will now be given. The turbidity of these sols containing 1 to 4 milli equivalent of K_4FeCy_6, coincides with the lowest relative viscosities of these sols. Microscopically this turbidity consists of a number of very sticky drops, easily flattened out to trails on a microscope slide. Thus, in the presence of tetra-valent ions a separation phenomenon can be realized in a strongly solvating medium (44 per cent acetone). The interpretation of this fact can be brought back to the discharging of a strongly positive sol by means of the tetravalent negative $FeCy_6$-ion, and the recharging to a negatively charged sol by the same ions. A more detailed discussion of this interesting case of separation can not be given here on account of the limited space. An indication of this recharging effect of the $FeCy_6$-ions can be found in the rise in the relative viscosity curve after the minimum. From measurements with K_2SO_4 it is evident that the bi-valent SO_4-ion is not able to recharge to a negative sol an originally strong positive sol

in 44 per cent acetone medium. In higher concentrations of K_2SO_4 the relative viscosity curve runs absolutely horizontal. The same holds good for KCl.

The effect of changing H-ion concentration on 44 per cent acetone gliadin sols with and without varying quantities of KCl (Table VI), must be discussed. Let us first consider the case of sols without electrolyte. By the addition of acid to a sol the H-ions of the acid will be preferably adsorbed. Part of the Cl-ions will be directed by the action of the H-ions and will form a double layer with these ions, while the rest of the negative ions move more or less freely in the surrounding medium. By increasing the acid concentration in such a sol the H-ion adsorption will likewise increase, but coupled herewith there will be an increase of the number of Cl-ions in the double layer. However, as a result of the increase of the Cl-ions in the double layer a decrease in hydration of the particle will take place. By these two contradictory forces the increase in charge (hydration) by the H-ions and the decrease in charge (hydration) by the Cl-ions, the maximum in the relative viscosity-acid concentration curve can be explained. In very high acid concentration the surface of the particle will be practically saturated with H-ions and the Cl-ion concentration in the medium will be so high that they are pressed into the double layer with the result that every H-ion will be faced by a Cl-ion. In aqueous medium with these effects of charge, is coupled the lyotropic effect of the electrolyte, but in the 44 per cent acetone medium used in our experiments these lyotropic effects have disappeared. On adding KCl to a sol of maximal charge, with increasing KCl concentration an increasing number of Cl-ions will be driven into the double layer, until the sol is practically discharged. In that case, just as in the case of a large excess of acid, every H-ion on the surface will be faced by a Cl-ion. These Hl and Cl-ions form a dipole on the surface of the particle and this dipole will bind solvate molecules. In the iso-electric state, where practically no H-ions are adsorbed on the surface, no dipoles can be formed and consequently the viscosity of such a sol must be lower than the, before mentioned, discharged sol. The viscosity level reached on discharging with KCl is practically the same as that reached with an excess of acid.

Finally, it should be emphasized that the discussion of the results presented here must be taken as an attempt to explain the observed phenomena. For this explanation it was necessary to introduce several suppositions. It is hoped, in due course, to test these suppositions, especially the composition of the solvation layer in alcohol and acetone media, and the adsorption of electrolytes in these media.

Summary

The following points were studied:

1. The influence of changing pH on the lyotropic effect in aqueous medium of the SO_4-ion in relation to the bi-valent discharging effect.

2. The influence of varying acetone concentration on acid-sols containing equimolar quantities of different mono-valent electrolytes. A reverse of the lyotropic series was observed in high concentrations.

3. The influence of increasing concentration of mono-valent electrolytes in the viscosity maximum in the acetone-water diagram.

4. The influence of poly-valent ions in 44 per cent acetone.

5. The influence of the acid concentration in the sol on the effect mentioned in items 3 and 6.

6. An explanation of the foregoing facts is given, based on the principle that there exists two kinds of adsorption: The one being an ion adsorption influencing the boundary potential, the other a molecular adsorption being the cause of the lyotropic effects.

7. In special alcohol or acetone media the last mentioned effect is the same or zero for electrolytes of the same valency. The differences between sols containing equivalent quantities of electrolyte of different valency can be brought back to a different state of charge of the particles.

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THE HEAT OF HYDRATION OF WHEAT FLOUR AND CERTAIN STARCHES INCLUDING WHEAT, RICE, AND POTATO [1, 2]

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(Read at the Convention, May, 1931)

Introduction

Accurate control of the temperature of the dough coming from the mixer is recognized in bakery practice as an important factor in the production of good bread. Several empirical formulae are in use for the calculation of dough temperature, but wide discrepancies are evident in the results obtained. As pointed out by Daniels, Kepner, and Murdick (1920), such a formula, to be accurate, must take into account the heat of hydration of the flour. The investigation reported in this paper further confirms the significance of this factor.

In a study, undertaken collaboratively by A. W. Alcock, of the Western Canada Flour Mills, Limited, Winnipeg, and this laboratory, of the role of moisture content in the rate of aging of flour mill streams, three grades of flour from the same mill-blend, each at moisture levels of approximately 8% and 14% were permitted to age in air-tight containers. When the baking test was applied to these flours it was noticed that the doughs made from the flours containing 8% moisture came from the mixer at temperatures 3° to 4° C. higher than doughs made from the same flours at the higher moisture level, when other conditions of mixing were similar. In their studies, Daniels, Kepner, and Murdick (1920) apparently took no cognizance of the moisture content of the flour used in their measurements which, in view of the above observation, casts some doubt on the validity of their conclusions. The present investigation was undertaken primarily to determine the influence of moisture content on the heat of hydration of wheat flour, but this was extended to include the influence of quantity and quality of protein, of heat treatment and particle size. Heats of hydration were also determined for wheat starches from various sources and for rice and potato starch.

Experimental

The heats of hydration were determined in an Emerson fuel calorimeter of the adiabatic type, thus avoiding cooling corrections.

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[2] Since this paper was prepared for publication, one on the same subject, by Emily Grewe, appeared in Cereal Chem. **8**: 162–165.

Preliminary experiments confirmed the observation of Daniels, et al., that the heat of hydration was practically independent of the relative quantities of flour and water. Two hundred grams flour or starch, on the dry matter basis, and 400 grams water was found to give satisfactory temperature changes and permitted effective mixing with a centrifugal type stirrer without the formation of internally dry agglomerates. The stirrer was driven by an electric motor of 0.25 h.p., the speed of which could be controlled by means of a rheostat in series with it. With the stirring speed selected, the heat of stirring was found to average 20 calories per minute in the instance of the wheat flour mixtures.

In order that a correction might be applied for the heat of stirring, experiments were conducted with a number of flour samples of varying moisture content with a view to determining the time necessary for the temperature of the mixture to reach a maximum after the addition of the flour to the water. It was found that two minutes provided ample time for the hydration to occur and the attainment of the maximum temperature, since for longer periods the subsequent rise in temperature was found to equal that resulting from stirring action alone. In subsequent experiments agitation of the flour-water mixture was continued for two minutes and 40 calories deducted from the total quantity of heat evolved during hydration.

Specific Heat of Wheat Flour

Since any thermal measurements necessitate a knowledge of the specific heats of the substances involved, it was necessary to obtain the value for the specific heat of wheat flour and of starch.

Jago [3] obtained values of 0.40 to 0.53 for the specific heat of flour in laboratory experiments, but he did not take into consideration the heat of hydration or the water equivalent of the apparatus used. On machine mixed doughs he obtained values of 0.30 to 0.45, again without cognizance of the heat of hydration, the heat absorbed by the mixer; or the heat generated during the process of mixing.

Daniels, Kepner, and Murdick (1920) determined the specific heat of wheat flour by packing 300 grams flour into small water-tight cylinders of negligible heat capacity, the whole being brought to a slightly elevated temperature in a thermostat. The cylinder and contents were then transferred to the calorimeter containing water at a known temperature, and the rise in temperature ascertained. This method gave 0.42 and 0.43 as the specific heat of flour containing about 13% moisture. They state:

"These values were checked by experiments in which the flour, at elevated temperatures was mixed directly with the water, allowance being made for the heat of hydration. By combining the data of two such experiments the specific heat was made the only unknown quantity."

[3] Cited by Daniels, Kepner and Murdick (1920).

The authors do not indicate the values obtained by this latter method, but state:

"The average of all the determinations of the specific heat of flour was taken as 0.43. As the moisture content of these flours was about 13% the specific heat of moisture-free flour would be about 0.34."

In view of the fact that the results reported above were expressed as approximations, and since it is conceivable that the specific heat may be different for different flours, it was decided to determine its value for flours of varying protein, ash, and moisture contents.

Method for Determining Specific Heat of Wheat Flour

In selecting a method, that outlined by Daniels, et al., involving the use of metal containers, was rejected on account of the low thermal conductivity of a material such as flour, although packing might obviate this to some extent. There are obvious objections, also, to the transfer of a warm body through air. The second method, in which the flour at elevated temperatures is mixed directly with the water, was rejected owing to the possibility of changes in the flour itself, or in its moisture content, on heating.

The method finally adopted gave promise of obviating the difficulties encountered in other methods. Sufficient flour to give 375 grams dry matter, and distilled water to bring the total weight of water to 825 grams, were placed in the calorimeter container, after having been thoroughly mixed to ensure uniformity in the flour-water distribution. The contents of the container were allowed to stand until no further rise in temperature was evident as a result of hydration of the flour. The mixture was then stirred in the calorimeter and 1000 grams distilled water at a known and slightly elevated temperature added from a well insulated glass container having an insulated delivery tube on the bottom through which the water could be permitted to flow into the calorimeter by means of a stopcock. The delivery was made through a small hole in the lid of the calorimeter. The rise in temperature of the mixture was ascertained and the specific heat calculated.

The values obtained for the specific heat using different flours did not differ appreciably. The average value, calculated for dry flour, was found to be 0.397 calorie per gram.

In the method adopted, the assumption is made that the specific heat of the water bound by the flour is the same as that of the unbound water. In any case, the amount of water held by the flour is relatively small as compared with the total amount of water present, and slight differences in the specific heats of the bound and free water—if such differences do exist—would, in all probability, cause a negligible error in the value for the specific heat.

Heat of Hydration of Wheat Flour

Method Used for Determining Heat of Hydration of Wheat Flour

The method finally adopted for the determination of the heat of hydration of flour was as follows:

Sufficient flour to yield 200 grams of dry matter was weighed out and placed in bottles of such shape that the contents could be quickly and completely removed by inverting the bottles. These were stoppered, sealed with paraffin, and placed in a thermostat maintained at approximately 25° C., in which they remained for at least six hours, usually over night. The flour was then found to have attained the temperature of the bath, ascertained with an error not exceeding 0.05° C. This method of obtaining the temperature of the flour was adopted after the futility of ascertaining it by inserting a thermometer into the flour itself became evident.

Into the calorimeter was put 400 grams distilled water, the thermometer inserted and the water stirred for a short time, after which its temperature was recorded. The flour was quickly added and the mixture stirred for two minutes; the temperature of the water in the surrounding jacket of the calorimeter being maintained as closely as possible to that of the contents. After two minutes the final temperature was recorded.

In the calculations of the heat of hydration, cognizance was taken of the water initially present in the flour, and of the heat of stirring. All values are expressed in calories per gram dry flour. Duplicate determinations usually agreed within 0.1 calorie per gram.

Influence of Moisture Content on the Heat of Hydration of Wheat Flour

For this study a sample of fifth middlings flour was selected, portions of which were brought to various moisture levels. Care was exercised to maintain conditions throughout the tempering process which would not be expected to cause changes in the flour itself.

Moisture determinations were made by the official vacuum oven method. The containers, into which the samples of flour at the various moisture levels were placed, were kept sealed with paraffin until the actual determinations of heats of hydration were to be made.

The results, recorded in Table I and represented graphically in Figure 1, show conclusively that the higher the moisture content of the flour, the lower the heat of hydration, but, as may be readily seen from Figure 1, there is not a linear relationship between the two.

TABLE I

RELATION BETWEEN MOISTURE CONTENT AND HEAT OF HYDRATION OF FIFTH MIDDLINGS FLOUR [1]

Moisture	Heat of hydration	Moisture	Heat of hydration
P.ct.	calories	P.ct.	calories
1.7	18.3	9.0	5.9
2.9	16.0	10.8	3.7
4.2	12.6	11.6	3.2
5.6	10.9	14.0	1.5
6.6	9.1	16.3	0.5
8.1	7.6		

[1] Heats of hydration expressed in calories per gram dry flour.

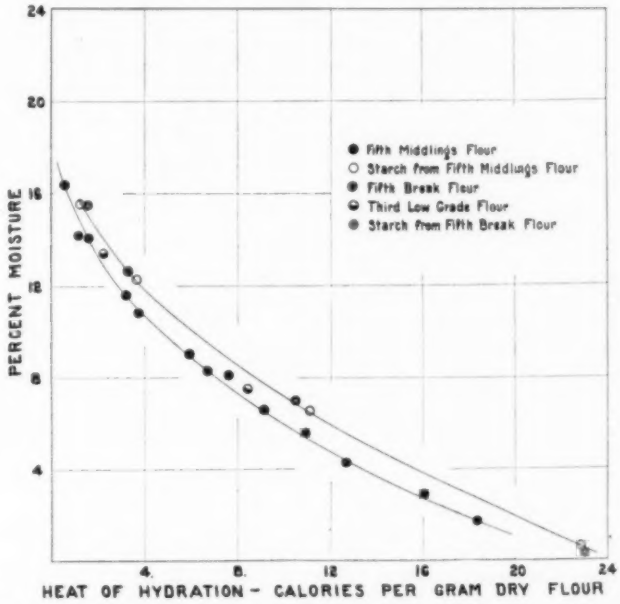

Fig. 1. The relation between the moisture content and heat of hydration of wheat flour; and the relative heats of hydration of wheat flour and wheat starch.

Influence of Quantity of Protein on Heat of Hydration of Wheat Flour

The material used for this study consisted of 5 straight grade flours, experimentally milled from hard red spring wheat with a range of 13.2% to 17.5% protein (dry matter basis), kindly supplied by R. K. Larmour of the University of Saskatchewan. Since the flours were all milled from sound wheat of the same variety grown in Saskatchewan in 1929, the quality of the protein was probably as uniform as one could expect to obtain with such a range in protein content.

Owing to the influence of moisture on the heat of hydration and the impossibility of conditioning different samples to the same moisture

content, it was necessary in an investigation of other factors to run a series of determinations at different moisture levels and plot curves to determine the effect of the variable under study.

Portions of each sample were conditioned to various moisture levels and the heats of hydration determined. The result are summarized in Table II and shown graphically in Figure 2. Figure 2

TABLE II

THE INFLUENCE OF PROTEIN CONTENT ON THE HEAT OF HYDRATION OF WHEAT FLOUR [1]

Per cent protein—Dry matter basis									
13.2		14.2		15.4		16.8		17.5	
Moisture	Heat of hydration	Moisture	Heat of hydration	Moisture	Heat of hydration	Moisture	Heat of hydration	Moisture	Heat of hydration
P.ct.	calories	P.ct.	calories	P.ct.	calories	P.ct.	calories	P.ct.	calories
14.0	1.4	14.4	1.0	14.3	1.2	14.5	1.1	13.8	1.5
12.2	2.4	12.6	2.3	12.6	2.3	12.5	2.4	12.4	2.7
10.1	4.8	10.1	4.6	10.1	4.5	10.1	4.5	10.1	4.5
8.0	7.2	8.0	7.2	8.1	6.9	8.2	7.0	8.1	7.2
3.4	14.5	3.4	14.8	3.3	14.9	3.3	14.6	3.4	14.5

[1] Heats of hydration expressed in calories per gram dry flour.

shows clearly that variations in the quantity of protein of similar quality, are not reflected in measurable differences in heats of hydration of wheat flour at similar moisture levels.

It was thought that differences in the heat of hydration as a result of variations in protein quantity might be revealed if lactic acid solution of suitable strength to bring the hydrogen ion concentration of the flour suspensions to pH 3.0, were substituted for pure water in the calorimetric determinations.

In several determinations in which lactic acid solutions were used, no detectable increment was observed in the heat evolved for the same flour, over that obtained when distilled water was the dispersion medium.

Influence of Protein Quality on the Heat of Hydration of Wheat Flour

Since variations in protein quantity of presumably similar quality had no determinable effect on the heat of hydration, it became of interest to determine the influence of protein quality. For this purpose, heats of hydration at several moisture levels were determined on four flours, three of which differed widely in origin and baking quality, and on three flour mill streams obtained from the same mill blend of hard red spring wheat.

In addition to this experimental material, in which any variations in protein quality were inherent, the possibility of changing the colloidal characteristics of the protein by heat treatment suggested itself.

Geddes (1930) has shown that heat treatment results principally in progressive impairment of gluten quality with increasing time and temperature of heating, and that the colloidal properties of starch are not appreciably altered by moderate heat treatment, as indicated by

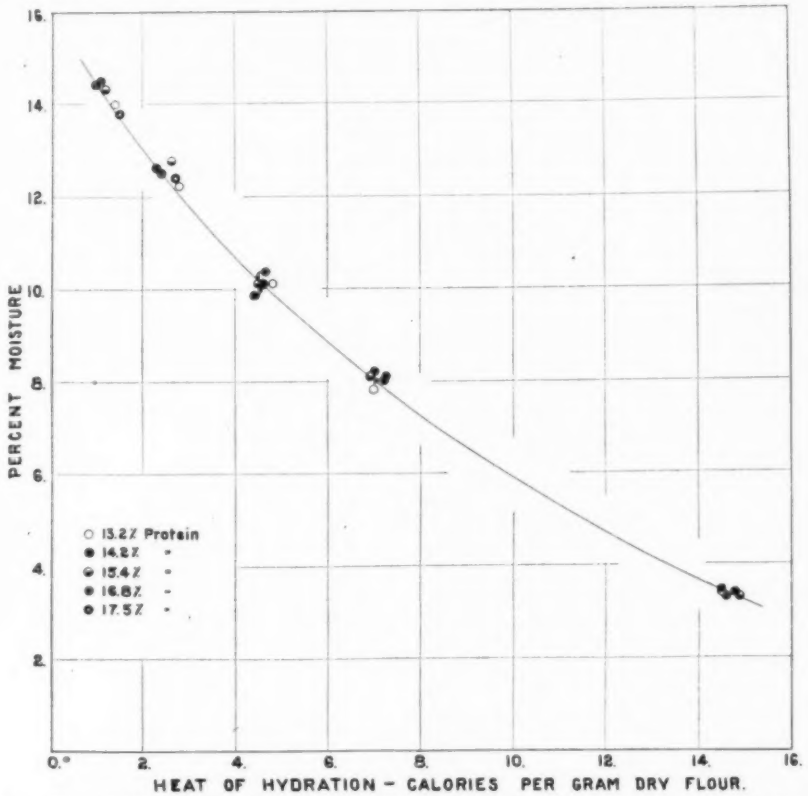

Fig. 2. The relation between the quantity of protein of similar quality, and the heat of hydration of wheat flour.

its resistance to the action of diastase. Accordingly, a first patent flour, milled from hard red spring wheat containing 15.1% protein (dry matter basis), was subjected to two heat treatments in the apparatus described by Geddes (1929). One portion was heated for 3 hours at 65° C., and the other for 10 hours at 80° C., and heats of hydration determined at five moisture levels in comparison with the unheated flour. As an indication of the extent of alteration in gluten quality, these samples were baked in duplicate according to the standard procedure, as outlined by Geddes and Winkler (1930), and the mean loaf volumes recorded.

The results obtained on the flours of varying origin are given in Table III, while the data for first patent flour subjected to heat treat-

ment are recorded in Table IV. These results are represented graphically in Figure 3. The experimental values obtained on the fifth break and third low grade mill streams have not been recorded, but the results are plotted in Figure 1, in comparison with fifth middlings flour.

TABLE III

THE EFFECT OF VARIATIONS IN PROTEIN QUALITY ON THE HEAT OF HYDRATION OF WHEAT FLOUR [1]

Percent protein—Dry matter basis							
Ontario winter		Kansas winter		Kansas winter		Pacific club	
10.8		17.6		16.6		8.7	
Moisture	Heat of hydration	Moisture	Heat of hydration	Moisture	Heat of hydration	Moisture	Heat of hydration
P.ct.	calories	P.ct.	calories	P.ct.	calories	P.ct.	calories
14.2	2.3	13.6	1.5	13.5	1.4	13.6	1.2
13.4	3.0	6.5	9.3	6.7	9.2	6.8	8.9
5.0	12.1	5.0	11.2	5.0	11.2	5.2	11.4

[1] Heats of hydration expressed in calories per gram dry flour.

TABLE IV

THE INFLUENCE OF HEAT TREATMENT ON THE HEAT OF HYDRATION OF FIRST PATENT FLOUR [1]

Control		Heat treatment			
		65° C.—3 hrs.		80° C.—10 hours	
Moisture	Heat of hydration	Moisture	Heat of hydration	Moisture	Heat of hydration
P.ct.	calories	P.ct.	calories	P.ct.	calories
16.3	0.3	16.7	0.0	15.9	0.2
13.7	1.9	13.1	2.1	12.3	2.5
11.0	3.5	10.8	3.9	11.0	4.0
5.1	11.7	5.1	11.4	5.0	11.1
3.8	14.6	3.7	13.9	3.7	13.6
Loaf volume 745 cc.		660 cc.		190 cc.	

[1] Heats of hydration expressed in calories per gram dry flour.

The curves given in Figure 3 indicate that protein quality does influence the heat of hydration although the differences are of lesser magnitude than might be expected. Heat denaturation resulting in decreased imbibitional capacity of the flour proteins is accompanied by a decrease in the heat obtained. The possibility of changes in the imbibitional capacity of the starch at the higher heat treatment cannot be excluded, but these changes are probably relatively slight compared with that undergone by the proteins. It is interesting to note that

practically identical values were obtained for the two Kansas flours of varying protein content. With regard to the flour mill streams, the data do not show appreciable differences between the heats of hydration of fifth break and fifth middlings flour, but the third low grade gave

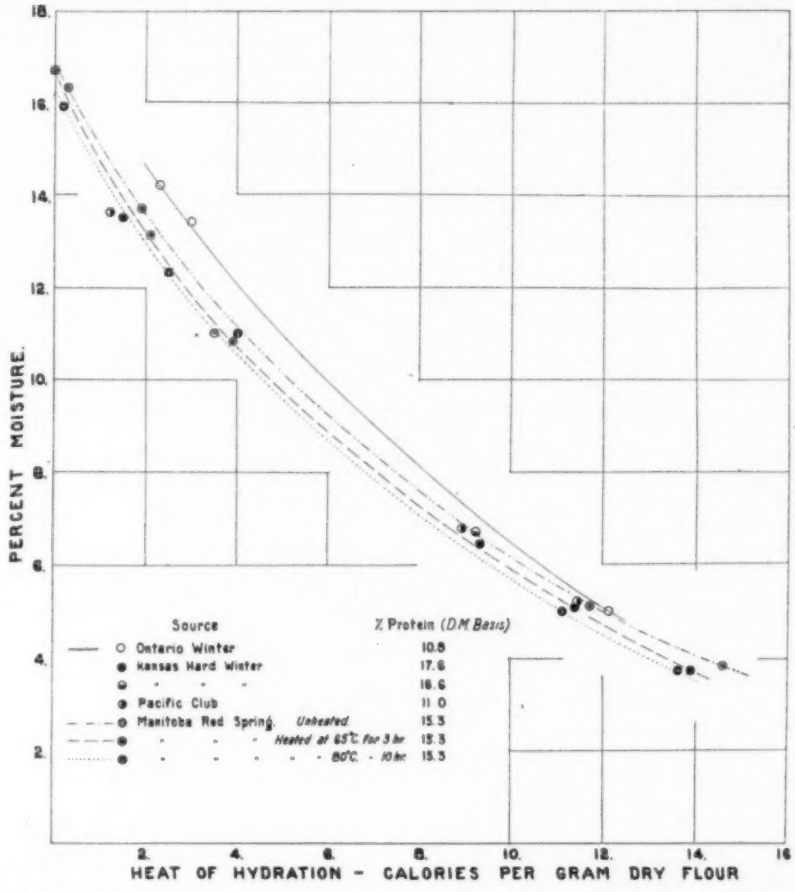

Fig. 3. The relation between the quality of protein and the heat of hydration of wheat flour.

considerably higher heats of hydration at comparable moisture levels. The protein content of the middlings, break, and third low grade flours were 14.2%, 26.0% and 15.6% on the dry matter basis respectively, and there is therefore no evident relation between protein content and the heat evolved during hydration. That differences in the starch present in the flours used are not responsible for the results obtained has been shown by determining the heat of hydration of starch prepared from these mill streams. The data are presented in the following section.

Specific Heat and Heats of Hydration of Wheat Starch

Since flours of varying origin yielded different heats of hydration it became of interest to determine to what extent the starch present was responsible for the values observed.

Padoa (1920) gives 0.308 calories per gram for the specific heat of potato and rice starch, but the method of determination is not stated. It was, therefore, decided to determine the specific heat of wheat, rice, and potato starch by the method adopted for the specific heat of flour. The values obtained for the three kinds of starch were in close agreement, the average value being 0.44 calories per gram dry starch. This value is considerably larger than that given by Padoa, but the difference is not sufficient to alter the values for heats of hydration to an appreciable extent.

To determine whether wheat starch from different sources would yield different values for heats of hydration at similar moisture levels, starch was prepared from flours according to the method outlined by Rask and Alsberg (1924). As an indication of the purity, protein and ash determinations were made. These values, together with the heats of hydration for the various moisture levels employed are given in Table V. The calorimetric data are graphically represented in Figure 1.

TABLE V

THE HEAT OF HYDRATION OF WHEAT STARCH [1]

Flour used as source of starch					
Fifth middlings		Fifth break		Pacific club	
Moisture	Heat of hydration	Moisture	Heat of hydration	Moisture	Heat of hydration
P.ct.	calories	P.ct.	calories	P.ct.	calories
15.7	1.2	15.3	1.5	14.6	2.3
12.2	3.6	12.6	3.3	13.0	3.3
6.5	11.1	6.9	10.5	7.2	10.1
0.6	22.8	0.3	22.9	—	—
Per cent ash (dry matter basis) 0.30		0.43		0.31	
Per cent crude protein (dry matter basis) 0.60		0.68		0.67	

[1] Heats of hydration expressed in calories per gram dry starch.

The results show quite conclusively that any variations observed in the heats of hydration of wheat flours at the same moisture level are not due to inherent differences in the starch present. It is also significant that the values obtained for wheat starch are considerably higher than for wheat flour, which probably accounts for the absence of a measurable influence of variations in the amount of protein on the

quantity of heat evolved. The heat of hydration of wheat flour is to a large extent due to the starch content, since the quantity of starch greatly exceeds the quantity of protein and the heat of hydration is also greater. Thus, relatively small variations in the quantity of protein present would hardly be expected to cause significant variations between different flours if the quality of the protein is similar. Furthermore, in view of the large contribution of starch to the total heat of hydration of wheat flour, differences in protein quality would not be reflected in widely differing values for the heat evolved from strong and weak flours.

Effect of Granulation on the Heat of Hydration of Wheat Flour

The observed differences in the heats of hydration of flours of varying origin might conceivably have been due to differences in granulation. The influence of this factor was investigated by running a series of calorimetric determinations on unground and ground highly refined middlings and on the flour obtained from the latter. This material was identical in chemical composition as indicated by protein and ash determinations. Table VI gives the relative sizes of the aggregates as determined by sieving. The results of the calorimetric determinations for various moisture levels are recorded in Table VII and graphically represented in Figure 4.

TABLE VI
RELATIVE SIZE OF FLOUR PARTICLES

Character of sample	Size of sieve—meshes per linear inch			
	70	109	124	124
	Per cent sample retained	Per cent sample retained	Per cent sample retained	Per cent sample passing
Unground middlings	5.9	45.3	35.4	13.4
Ground middlings	1.6	21.4	37.4	39.6
Flour	0.0	0.0	0.0	100.0

It is evident from Figure 4 that the extent to which the component particles of wheat flour are aggregated has no determinable effect on the heat of hydration within the range of size studied. That this should be the case is rather to be expected, regardless of the manner in which the water is held by the flour.

If it is assumed that the effect is solely on the surface of the particles constituting the aggregates, the heat of hydration would be independent of the size of the aggregates, within certain limits, since the interfacial areas of the component particles are practically unaltered.

Again, if the assumption is made that the water is dispersed throughout the particles of which the aggregates are composed, the total mass of dry flour present determines the amount of water held, and consequently the amount of heat evolved.

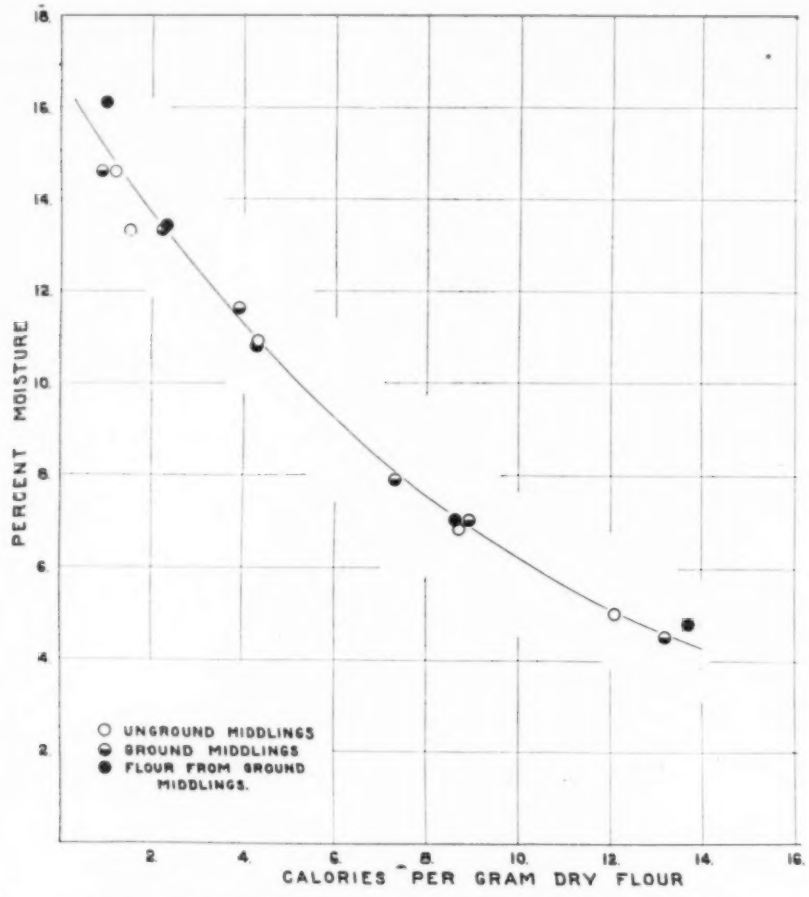

Fig. 4. The influence of particle size on the heat of hydration of wheat flour.

In either case, diffusion of the water would be a factor, and it is logical to expect a decrease in the velocity of hydration, which should be reflected in an increased length of time necessary to attain the maximum temperature, unless diffusion is very rapid. The rapidity with which the increment in temperature occurred was the same in all cases, as nearly as observations were able to disclose. This leads to the conclusion that diffusion of the water throughout the aggregates, or into the individual particles must occur very rapidly.

In an effort to determine whether the water of hydration was held

by the flour solely on the surface of the individual particles or whether the water permeated these particles, starch was substituted for flour. The starches of rice and potato were selected as representative of very small and large particle sizes respectively. Potato flour, on analysis, proved to be practically pure starch, containing 0.30% ash and 0.56% crude protein, on a dry matter basis, and was used without further purification. Rice starch was prepared from rice flour by the method outlined by Larmour (1927), and was found to contain 0.58% ash, and 1.34% crude protein, on a dry matter basis.

TABLE VII

THE EFFECT OF GRANULATION ON THE HEAT OF HYDRATION [1]

Unground middlings		Ground middlings		Flour	
Moisture	Heat of hydration	Moisture	Heat of hydration	Moisture	Heat of hydration
P.ct.	calories	P.ct.	calories	P.ct.	calories
5.0	12.1	4.5	13.2	4.8	13.7
6.8	8.7	7.0	8.9	7.0	8.6
10.9	4.3	7.9	7.3	10.8	4.3
13.3	1.5	11.6	3.9	13.4	2.3
14.6	1.2	13.3	2.2	16.1	1.0
—	—	14.6	0.9	—	—

[1] Heat of hydration expressed in calories per gram dry flour.

The results of the calorimetric determinations for the moisture levels used are shown in Table VIII and represented graphically in Figure 5. Values for the heat of hydration of wheat starch at various moisture levels, as previously determined, are also plotted for purposes of comparison.

TABLE VIII

THE HEAT OF HYDRATION OF RICE AND POTATO STARCH [1]

Rice starch		Potato starch	
Moisture	Heat of hydration	Moisture	Heat of hydration
P.ct.	calories	P.ct.	calories
1.03	20.6	0.8	24.2
6.6	9.9	6.6	14.0
13.6	1.4	17.0	2.4
15.3	0.2	18.0	1.7

[1] Heat of hydration expressed in calories per gram dry starch.

The regularity of the trend of the results in each case leaves no doubt that the heat of hydration of potato starch is much greater than that of rice at the same moisture level. From the relative positions of the three curves, the conclusion might be drawn that the smaller the

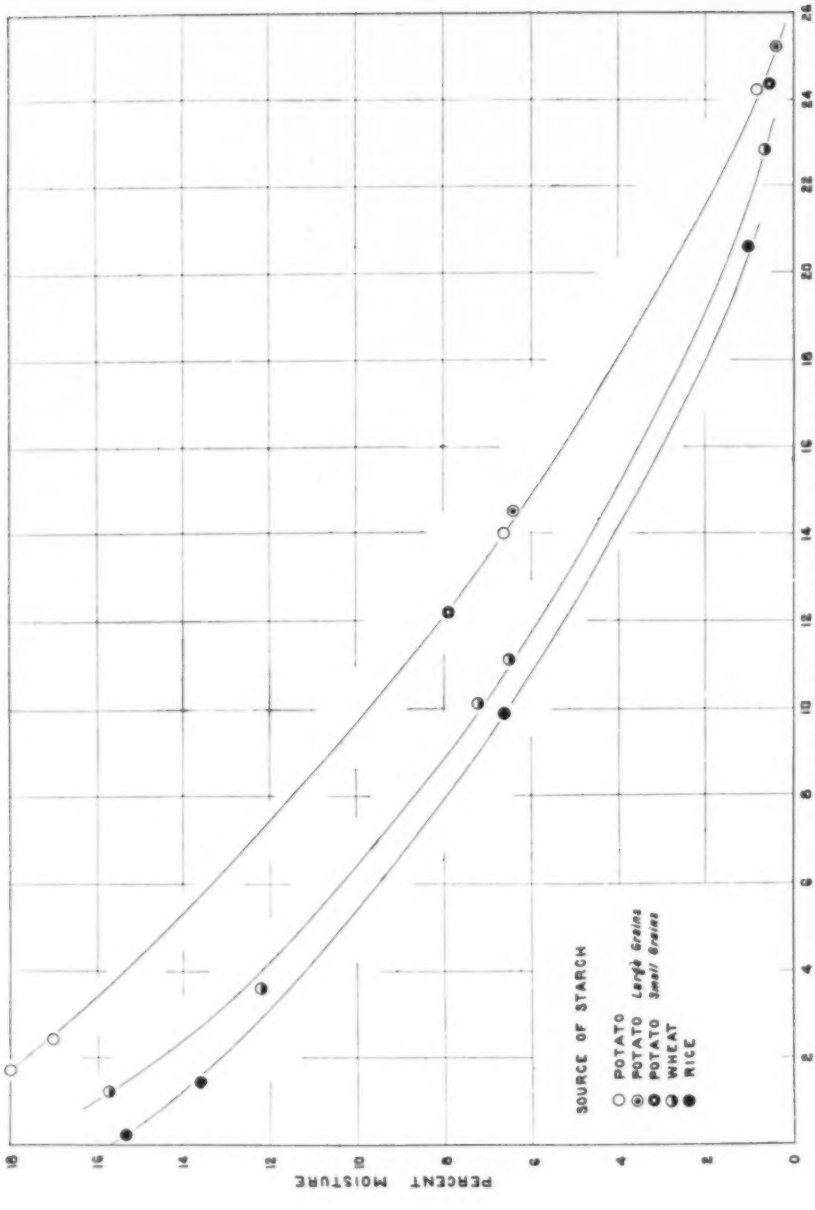

Fig. 5. The relative heats of hydration of starch of rice, wheat, and potato.

size of the particles, the less the heat of hydration. However, the possibility of inherent differences in the starches from the various sources cannot be neglected. Consequently, a further investigation into the problem was made.

Potato starch provided a source from which particles of very different sizes, but possessing the same inherent characteristics, might be obtained. After much preliminary work, a Nobel soil elutriator was finally employed to effect the fractionation of the starch. Although very tedious and time consuming, elutriation provided two fractions differing widely and uniformly in the size of the particles.

The average diameter of each fraction, as determined by randomized microscopic measurements, was found to be 0.01 mm. and 0.07 mm. for the fine and coarse particles respectively. These values are, of course, approximate.

Portions of each fraction were brought to different moisture levels, and the corresponding heats of hydration determined. The results are shown in Table IX and plotted in Figure 5.

TABLE IX

INFLUENCE OF PARTICLE SIZE ON THE HEAT OF HYDRATION OF POTATO STARCH [1]

Average size of particle = 0.01 mm.		Average size of particle = 0.07 mm.	
Moisture	Heat of hydration	Moisture	Heat of hydration
P.ct.	calories	P.ct.	calories
0.54	24.3	0.35	25.2
7.9	12.2	6.4	14.5

[1] Heat of hydration expressed in calories per gram dry starch.

Although only two moisture levels were obtained, owing to the difficulties encountered in fractionation of the starch, the results give no indication whatever of increased heat of hydration with decrease in the size of the individual particles, which corresponds, of course, to an increase in total surface area per unit of weight.

From these observations, taken in conjunction with the data obtained for the relative values for the heats of hydration of rice, wheat, and potato starches, it must be concluded that the extent of the surface area alone does not determine the magnitude of the quantity of heat evolved, but that the nature of the material undergoing hydration is a determining factor.

Discussion

The assumption that the heat evolved, when flour and water are mixed, is a consequence of true and simple adsorption, seems to offer the most satisfactory explanation for the results obtained.

Evidence in support of this postulate was readily obtained by a simple mathematical manipulation of the data in Table I. Taking 1.7% moisture, corresponding to 18.3 calories per gram of dry flour, as the base from which to calculate the increment in heat corresponding to a given increment in grams of water taken up by the flour, the results shown in Table X are obtained.

TABLE X

RELATION BETWEEN THE INCREMENTS IN THE HEAT OF HYDRATION OF, AND WATER TAKEN UP BY FIFTH MIDDLINGS FLOUR.[1]

Moisture	Heat of hydration per gm. (d.m. basis)	Water imbibed per gm. flour	Heat evolved per gm. flour	$dQ/d(H_2O)$
P.ct.	calories	Grams	calories	
1.7	18.3	—	—	—
2.9	16.0	0.012	2.3	191.7
4.2	12.6	0.025	5.7	228.0
5.6	10.9	0.039	7.4	189.7
6.6	9.1	0.049	9.2	187.8
8.1	7.6	0.064	10.7	167.2
9.0	5.9	0.073	12.4	169.9
10.8	3.7	0.091	14.6	160.4
11.6	3.2	0.099	15.1	152.5
14.0	1.5	0.123	16.8	136.6
16.3	0.5	0.146	17.8	121.9

[1] Data for flour containing 1.7% moisture taken as base.

If now the increments in the grams of water taken up by one gram of flour, referred to the base 0.017 gram water per gram flour, are plotted as abscissae and the corresponding increments in the heat of hydration, referred to the base 18.3 calories per gram dry flour at the moisture content of 1.7%, as ordinates, the curve shown in Figure 6 is obtained.

The curve is evidently of the same type as the adsorption isotherm. To ensure the identity of the types, it is necessary that the logarithms of the increments in the amount of water taken up by the flour should be proportional to the logarithms of the increments in the heat of hydration.

The straight line in Figure 6 represents the proportionality existing between these quantities, as determined from the curve, while the actual proportionality is shown by the distribution of the calculated values for the logarithms about this line.

The results dispel any doubt which may exist regarding the cause of the heat observed when flour and water are mixed. Adsorption of a simple nature occurs which, since the adsorbed substance is water, may be termed hydration. It is known that this phenomenon is accompanied by a decrease in the volume of the system, due possibly

to orientation of the molecules on the surface of the micelles, and to the imbibition pressure exerted. The heat developed is believed to be a consequence of this pressure, which may be of great magnitude, as shown by Harkins and Ewing (1921), and others.

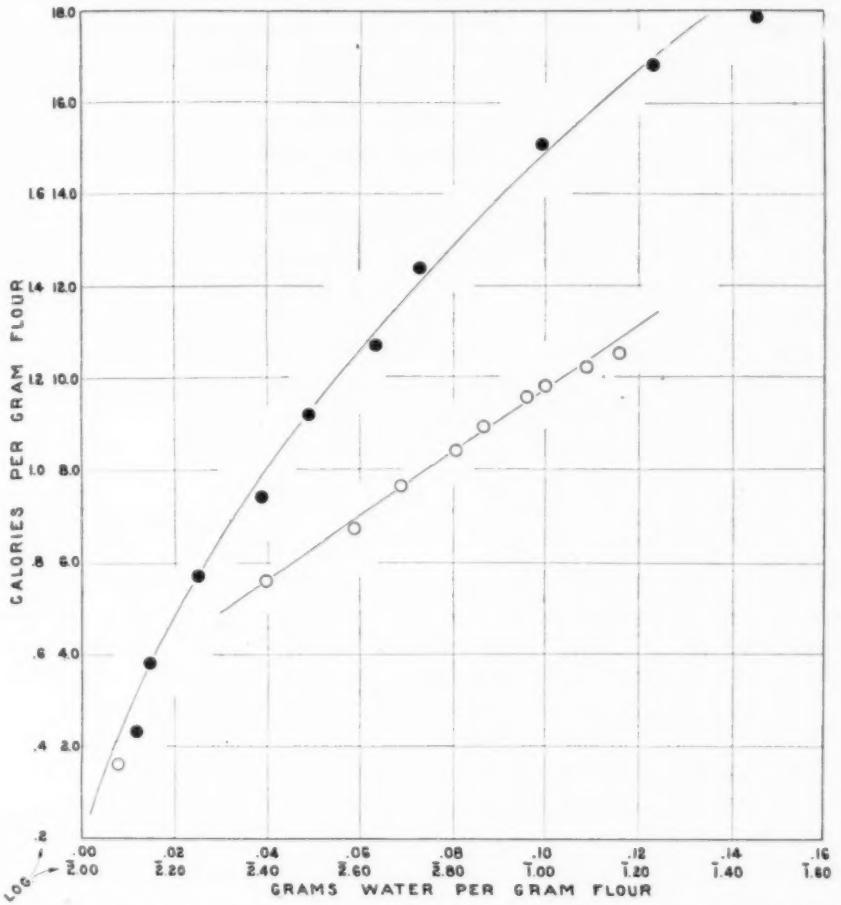

Fig. 6. The curve shows the relationship existing between the increments in grams of water taken up by the flour, and the corresponding increments in the heat evolved, expressed in calories per gram dry flour. The straight line represents the proportionality existing between the logarithms of these values as obtained from the curve.

The data obtained for the heats of imbibition of rice, wheat, and potato starch, would seem to point undeniably to the conclusion that the water permeates the starch granules, and is adsorbed on the surfaces of the micelles constituting the granule. Investigations reported in a series of splendid papers by Alsberg (1926), Alsberg and Griffing (1925), and Alsberg and Perry (1924), on the colloidal properties of starch and flour, suggest that the greater heat of imbibition of

some starches than others may be due solely to mechanical effects. The imbibitional pressure developed is probably a function of the amount of water imbibed. The larger particles may be expected to hold more water, if permeation of the granule by the water is admitted. This would mean a greater pressure per unit area of surface in the granule, which, in turn, would correspond to increased heat of imbibition. These considerations, in conjunction with the possibility of inherent differences in the starches studied, may account for the unexpected results obtained.

Aside from the theoretical aspects indicated above, the results are also of some practical significance.

From the point of view of commercial bakery practice, they emphasize the necessity of considering the influence of variations in moisture content of flour on the heat of hydration developed in the mixer, as this is an important factor in determining the temperature of the resulting dough. In experimental baking, it is the usual practice to bring the flours to a uniform temperature and, by a few trial mixings, determine the temperature of the solutions used to bring the doughs out at the desired temperature. It is obvious that such control may yield doughs of varying temperature if the moisture content of the several flours baked is not uniform. Milling practice in this laboratory involves conditioning the wheat samples to a uniform moisture content three days prior to the final tempering. The mill room is equipped with apparatus for humidity control and the flour obtained is placed in sacks and aged in the mill room. This procedure yields flours of practically constant moisture content.

They may also serve to explain the difficulties encountered in determining the moisture content of flour and allied substances. Snyder and Sullivan (1924, 1925, 1926), Nelson and Hulett (1920), and others have shown that the complete removal of the water from such material is attended with considerable difficulty, the amount of moisture obtained depending to a large extent on the method employed.

It has been shown that the quantity of heat liberated during hydration of wheat flour and similar substances increases to a maximum as the moisture content decreases. Conversely, as the moisture content decreases, the quantity of heat required to remove the residual moisture must increase.

Neuhausen and Patrick (1921) have shown that a silica gel, heated to 300° C. in a vacuum for 6 hours retains 4.8% moisture. It does not follow that the same would be true for flour, although the heats of adsorption of the two are of the same order, since the nature of the adsorbent determines the force with which the water is held. How-

ever, it is probable that the difficulties encountered in moisture determinations may be explained in this way.

Summary

A study has been conducted of the factors influencing the heat of hydration of certain bio-colloids, including wheat flour and the starch of wheat, rice, and potato.

A method was also elaborated for determining the specific heat of wheat flour and the starches used in this study, the average values obtained being 0.397 and 0.44 calories per gram respectively, expressed on the dry matter basis.

The heat of hydration varied widely, depending upon the moisture content. For example, a fifth middlings flour milled from hard red spring wheat, containing 1.7% moisture evolved 18.3 calories per gram dry matter on hydration, while at 16.3% moisture the heat of hydration was only 0.5 calories per gram. The relation between moisture content and heat of hydration is not linear.

Calorimetric determinations on 5 straight grade flours, milled from hard red spring wheat grown in Saskatchewan and ranging in protein content from 13.2% to 17.5% (dry matter basis) gave essentially the same heats of hydration at similar moisture levels. This observation is in harmony with the observations of Coleman and Fellows (1925), that wide variations in protein content exerted no influence on the hygroscopicity of wheat. The heats of hydration were not altered by using lactic acid solution of sufficient strength to bring the flour suspension to pH 3.0 in place of distilled water in the calorimeter.

Variations in protein quality were reflected in only slight differences in the heat of hydration. In addition to determinations on flours showing inherent differences in protein quality, heat treatment was resorted to as a means of altering the colloidal character of the flour proteins. At comparable moisture levels flour milled from Ontario winter wheat gave the highest values and a strong flour subjected to severe heat treatment the lowest at comparable moisture levels. The differences observed, however, were relatively slight compared with the variations in baking quality. In this connection it is of interest to note that Newton and Cook (1930) found no significant differences in the hydration of strong and weak flours by the bound-water method. Our data are in agreement with their belief that such differences that may exist are too small to be of major importance as factors in baking quality.

Wheat starches prepared from different flours gave essentially the same heats of hydration which were somewhat higher than for wheat flour at the same moisture content.

Variations in the average size of the flour aggregates were not reflected in variations in the heat of hydration.

The heats of hydration of the starch of rice, wheat, and potato starches were found to differ widely at the same moisture content, and to increase in the order mentioned. However, when potato starch was separated into two fractions consisting of large and small granules respectively, there was no significant difference in the heats of hydration.

The conclusion is reached that hydration is not an external surface effect alone, and that the water premeates the starch granule. The nature of the starch was also recognized as an influential factor.

The evolution of heat observed when water is mixed with wheat flour or starch has been shown to be due to adsorption, and this adsorption is not complex.

The practical significance of the results, from the point of view of bakery practice and the determination of the moisture content of bio-colloids, is indicated.

Acknowledgments

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A SIMPLE METHOD FOR DETERMINING THE ASH CONTENT OF THE FLOUR IN SELF-RISING AND PHOSPHATED FLOURS[1]

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Introduction

Logue and McKim (1931) quote the figures of LeClerc and Bailey, Pope, and the Bureau of Census to show that self-rising flour now constitutes approximately 10% of all the flour consumed in this country and 21.7% of all the flour consumed by the housewife. They further point out that the consumption of self-rising flour is increasing more rapidly than that of plain or natural flour.[2] No definite figures as to the amount of phosphated flour consumed are available, but Heywood (1931) estimates that slightly more of it is produced than self-rising flour, the production of the latter being approximately 9,000,000 barrels annually as calculated from the amount of soda sold, while the estimated production of phosphated flour is 10,000,000 barrels.

People interested in these flours have long sought for a simple method for accurately determining the grade of flour used in their manufacture. Baking and slick tests give some idea of their grade but at best such tests are only approximate. Bailey (1925) reviewed the various methods that have been used to determine flour grade. He states that ash content is the most extensively used criterion of

[1] This work was supported by a Fellowship from The National Soft Wheat Millers' Association.
[2] Flour without the addition of phosphates, or self-rising flour ingredients.

flour grade, except possibly color and visual appearance. In self-rising flour, the ash content has been greatly increased by the addition of chemical leavening agents and salt and accordingly can not be used as an index of its grade. Likewise, the ash content of phosphated flour has been modified by the addition of phosphate.

However, if it was possible to remove these added ingredients quantitatively without affecting the ash content of the flour itself, then the ash content of the flour could be determined and the grade of flour indicated.

Experimental

For a long time cereal chemists have used, among other tests, a very simple test to determine whether or not a particular flour is plain or phosphated. The test is performed in a test tube by shaking a few grams of flour with carbon tetrachloride. Due to differences in specific gravity, the phosphate will settle at the bottom of the test tube while the flour rises to the surface. It occurred to the author that possibly this separation of added ingredients could be made quantitatively. Accordingly, a few samples of phosphated and self-rising flours were mixed with carbon tetrachloride in large funnels fitted with pinchcocks. The mixtures were stirred from time to time and after several hours, the flour was skimmed off, air-dried, and the moisture and ash content determined in the usual way. The ash results were much too high indicating that the separation had been incomplete. The use of a centrifuge next suggested itself as a means of making the separation more complete, and after a number of trials, the following method was developed, with minor modifications for different classes of flour, for determining the ash content of the original flour in self-rising and phosphated flours.

Method and Materials Used

Weigh and transfer 20 to 25 grams of flour to a metal centrifuge tube cup 2 inches in diameter and 6 inches deep. Add sufficient carbon tetrachloride to fill the centrifuge tube cup to about an inch from the top (about 250 cc. is required). Centrifuge the mixture in an International Electric Centrifuge—size 2, for 5 to 7 minutes at a speed of 1600 R.P.M. Allow the centrifuge to come to rest slowly. Carefully skim off the flour, which is now in a compact layer on the surface of the carbon tetrachloride, with a large tablespoon, recovering as much of the flour as is possible in one spoonful. With care about 90% of the original flour can be recovered. Allow the wet flour to air dry over night. Determine moisture and ash in the usual way. The carbon tetrachloride remaining may be filtered, distilled, and used again. Reduce the ash content of the plain (control) flour and the ash content of the flour recovered from the self-rising flour to a uniform moisture basis in order to make direct comparisons.

As material with which to study the efficiency and utility of the method with respect to the determination of the original ash content of self-rising flour, 73 samples representing different grades of hard and soft wheat plain flour were obtained from mills located in several states. Fifty-three of these were soft wheat flours, while 20 were hard wheat flours. The samples were divided into two parts, one part was made into self-rising flour, while the other part served as the control. The self-rising soft wheat flours, as well as the control flours, were in storage for about six months before use, because the soft wheat flours were originally prepared for use in another investigation. The self-rising flours made from the hard wheat flours were used soon after being mixed.

Results with Self-Rising Flour

The results of the test made with the self-rising soft wheat flours are given in Table I.

With respect to the data obtained from the analysis of the self-rising soft wheat flours, it will be seen that in no case was the difference in ash content between the plain (control) flour, and the flour recovered

TABLE I

THE ASH CONTENT OF PLAIN SOFT WHEAT FLOUR COMPARED TO THE ASH CONTENT OF THE SAME FLOUR AFTER REMOVAL FROM SELF-RISING FLOUR

Sample number	Ash content [1] of plain flour	Ash content of flour [1] removed from self-rising flour	Difference
	Pct.	Pct.	Pct.
1	0.333	0.339	0.006
2	.411	.424	.013
3	.524	.527	.003
5	.408	.430	.022
6	.439	.461	.022
8	.404	.416	.012
9	.549	.530	.019
10	.679	.659	.020
12	.388	.391	.003
13	.430	.412	.018
14	.481	.470	.011
16	.384	.397	.013
17	.406	.394	.012
18	.680	.684	.004
20	.361	.342	.019
21	.398	.391	.007
22	.422	.401	.021
23	.512	.491	.021
25	.382	.391	.009
26	.425	.445	.020
27	.574	.572	.002
29	.431	.420	.011
31	.345	.343	.002
32	.378	.392	.014
33	.525	.511	.014
35	.358	.366	.008

TABLE I—*Continued*

36	.492	.491	.001
37	.519	.506	.013
39	.373	.375	.002
40	.428	.427	.001
41	.524	.519	.005
43	.375	.378	.003
44	.422	.430	.008
45	.525	.524	.001
47	.335	.346	.011
48	.414	.427	.013
49	.459	.450	.009
51	.362	.373	.011
52	.418	.415	.003
53	.619	.596	.023
55	.507	.500	.007
57	.340	.350	.010
58	.418	.431	.013
59	.466	.475	.009
60	.371	.373	.002
61	.477	.463	.014
62	.509	.513	.004
63	.374	.379	.005
64	.400	.410	.010
65	.738	.735	.003
67	.423	.426	.003
68	.516	.518	.002
69	.565	.575	.010

Average difference in ash of plain flour and ash of original flour in self-rising flour	0.0098%
Maximum difference in ash of plain flour and ash of original flour in self-rising flour	.023 %
Minimum difference in ash of plain flour and ash of original flour in self-rising flour	.001 %

[1] All ash results are on the basis of 15% moisture.

from the self-rising flour (the original flour) greater than .023%. The average difference for the 53 samples was 0.10%. The latter figure is well within the limits of experimental error of an ash determination.

The same method was then applied to a few samples of self-rising hard wheat flours, the data from the analysis of which are shown in Table II. The results were low, indicating that some of the high ash flour particles had either been carried down with the self-rising ingredients during centrifuging or were held in suspension in the carbon tetrachloride. However, by reducing the speed of the centrifuge to 1100 R.P.M. and allowing the centrifuged material to stand 30 minutes before skimming off the flour, results in agreement with those obtained with soft wheat flours were obtained except in cases where the ash content of the sample was above 1%. However, by definition, these latter samples do not come under the classification of flour, but rather of feed.

We know by observation that hard wheat flours are much more granular than are soft wheat flours. Also, hard wheat flours have a higher specific gravity. These facts may help to explain why it is necessary to modify the procedure used on soft wheat flours to make the method applicable to hard wheat flours.

TABLE II

THE ASH CONTENT OF PLAIN HARD SPRING, HARD WINTER, AND HARD WHITE WHEAT FLOUR COMPARED TO THE ASH CONTENT OF THE SAME FLOUR AFTER REMOVAL FROM SELF-RISING FLOUR

Sample number	Ash content [1] of plain flour	Ash content flour in self-rising flour	Difference
	Pct.	Pct.	Pct.
200	0.384	0.397	0.013
201	.643	.655	.012
202	1.110	1.060	.050
203	.422	.434	.012
204	.622	.619	.003
205	.806	.833	.027
206	1.160	1.070	.090
207	.427	.424	.003
208	.358	.382	.024
209	.358	.378	.020
210	.385	.389	.004
211	.332	.324	.008
212	.429	.405	.024
213	.417	.426	.009
214	.399	.400	.001
215	.392	.374	.018
216	.455	.426	.029
217	.483	.497	.014
218	.500	.497	.003
219	.445	.447	.002

Average difference in ash of plain flour and ash of original flour in self-rising flour	0.037%
Maximum difference in ash of plain flour and ash of original flour in self-rising flour	.090%
Minimum difference in ash content of plain flour and ash content of original flour in self-rising flour	.002%

[1] Ash results on the basis of 15% moisture.

Results with Phosphated Flours

For the study of the application of the method to the determination of the ash content of the original flour in phosphated flours 20 samples of phosphated soft wheat flour and 10 samples of phosphated hard wheat flour were used. The analytical data are shown in Table III.

In the case of phosphated flours, it was found that centrifuging at a speed of 800 R.P.M. for $2\frac{1}{2}$ minutes was sufficient to remove the phosphate. After centrifuging, two procedures were followed: (1) the carbon tetrachloride flour mixture was allowed to stand for 30 minutes before skimming off the flour, and (2) the mixture was allowed to stand for 7 to 16 hours before skimming off the flour. In both cases, the ash content of the flour removed from phosphated samples of flour was lower than that of the plain flour samples. By allowing the mixture to settle 30 minutes before skimming, the average difference in the ash contents of the plain flour and the flour removed from phosphated soft wheat flours was $-.032\%$, while by the use of the longer period of

TABLE III

THE ASH CONTENT OF PLAIN SOFT WHEAT FLOURS COMPARED TO THE ASH CONTENT OF THE SAME FLOURS AFTER REMOVAL FROM PHOSPHATED FLOUR

Sample number	Ash content of plain flour [1]	Ash content original flour in phosphated flour by procedure		Difference by procedure		Corrected ash values for procedure		Corrected difference for procedure	
		I	II	I	II	I	II	I	II
	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
300	0.333	0.293	0.323	0.040	0.010	0.325	0.348	0.008	0.015
301	.406	.380	.410	.026	.004	.412	.435	.006	.029
302	.447[2]	.385	.401	.062	.046	.417	.426	.030	.021
303	.440	.397	.425	.043	.015	.429	.450	.011	.010
304	.389	.361	.375	.028	.014	.393	.400	.004	.011
305	.385	.349	.357	.036	.028	.381	.382	.004	.003
306	.428	.398	.384	.030	.044	.430	.409	.002	.019
307	.423	.411	.385	.012	.038	.443	.410	.020	.013
308	.520[2]	.477	.463	.043	.057	.509	.485	.011	.035
309	.443	.403	.428	.040	.015	.435	.453	.008	.010
310	.322	.303	.319	.019	.003	.335	.344	.013	.022
311	.380	.349	.370	.031	.010	.381	.395	.001	.015
312	.475	.454	.463	.021	.012	.486	.488	.011	.013
313	.509	.493	.494	.016	.015	.525	.519	.016	.010
314	.379	.343	.352	.036	.027	.375	.377	.004	.002
315	.376	.345	.354	.031	.022	.377	.379	.001	.003
316	.493[2]	.458	.434	.035	.059	.490	.459	.003	.034
317	.437	.391	.405	.046	.032	.423	.430	.014	.007
318	.690[3]	.621	.636	.069	.054	.698	.693	.008	.003
319	.739[3]	.667	.678	.072	.061	.737	.735	.002	.004

Average difference in ash of plain flour and ash in original flour of the phosphated flours (Procedure I)—0.032%.

Average difference in ash of plain flour and ash in original flour of the phosphated flours (Procedure II)—0.025%.

[1] Ash content is on the basis of 15% moisture.
[2] Bleached heavily with Novadelox.
[3] Samples 318 and 319 were not included in the above averages.

standing, the average difference was −.025%. With low grade flours containing about 0.70% ash the differences were greater, −.070% and −.057% respectively. If the ash results on the phosphated flours are corrected by adding the average difference, the ash will be within ± 0.02% of the ash of the plain flour.

One half of the plain soft wheat flours had been treated with Novadelox which would increase the ash slightly. Samples number 302, 308 and 316 were treated heavily with Novadelox as was determined by shaking 25 grams of flour with carbon tetrachloride in a Goetz tube and noting the amount of sediment deposited.

Only the second procedure was used on the hard wheat phosphated flours. The data are shown in Table IV. The results were somewhat better than those of the soft wheat samples, the average difference

TABLE IV

THE ASH CONTENT OF PLAIN HARD WHEAT FLOUR COMPARED TO THE ASH CONTENT OF THE SAME FLOURS AFTER REMOVAL FROM PHOSPHATED FLOUR

Sample number	Ash content plain flour	Ash content flour in phosphated flour. Procedure II	Difference	Corrected ash phosphated flours	Corrected difference
	Pct.	Pct.	Pct.	Pct.	Pct.
320	0.364	0.350	0.014	0.360	0.004
321	.626	.584	.042	.594	.032
322	.399	.398	.001	.408	.009
323	.600	.578	.022	.588	.012
324	.785	.772	.013	.782	.003
325	.405	.415	.010	.425	.020
326	.343	.348	.005	.358	.015
327	.396	.394	.002	.404	.008
328	.390	.392	.002	.402	.012
329	.491	.467	.024	.477	.014
Average difference in ash of plain flour and ash in the original flour of phosphated flour					.010

between the ash content of the plain and phosphated flours being -0.010%.

In almost every case the ash results on the flours recovered from the phosphated samples were lower than those of the plain flours. This was not true of the self-rising flours. With the self-rising flours it is believed that the loss of ash due to suspension in the carbon tetrachloride of some of the higher ash flour particles and to the presence of foreign material such as dirt in the flour and to Novadelox, may be compensated for by the retention of traces of the self-rising ingredients in the flour during the centrifuging process.

It has occurred to the author that it may be possible to remove the self-rising ingredients by using a lower speed of centrifuging than employed in this study and also that separatory funnels might be substituted for centrifuge cups but these problems have not been investigated.

Summary

In conclusion a simple method is described for determining the original flour ash content of self-rising and phosphated flours.

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THE CHEMICAL ANALYSES OF SOME IMPORTANT BAKING INGREDIENTS

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Introduction

Practically all ingredients which are used in the modern bakeshop have an important influence upon the quality of the finished product. The progressive bakeshop superintendent of today demands and uses only tested materials which have been manufactured under careful chemical control. The large baking corporations have well equipped laboratories with a competent staff of chemists, and the independent baker subscribes to scientific service or submits samples of ingredients to commercial laboratories for testing.

Flour is, of course, by far the most important ingredient that the baker uses and volumes have been written on the testing of this material alone. The standardization of methods and specifications for testing flour, however, has been referred to a special committee. This report, therefore, will not touch upon this very important subject.

Other baking materials have a marked influence on the quality of the bakers' products or upon optimum conditions of fermentation, manipulation, or handling. Yeast foods, bread improvers, and flour improvers are a few of the materials that the baking chemist is called upon to analyze and investigate.

The average cereal chemist is not very familiar with the testing of these products and approved methods of examining them should prove valuable and of interest to him. This report will, therefore, attempt a detailed description of determinations which are helpful in characterizing these products and determining their value. The methods given are those employed in the Analytical Laboratory of The W. E. Long Company, and were developed and collected through years of research in an effort to give their baker-clients reliable information regarding these products, to aid them in making satisfactory purchases and to help them in adjusting their production methods.

Yeast foods, bread and flour improvers usually consist of a complex mixture of organic materials and mineral salts. Ammonium compounds and salts of calcium, magnesium and potassium are often combined with oxidizing agents in a mixture, and salt and cereal products are often present in large quantities as diluents or fillers. The common ingredients in yeast foods and improvers are as follows: calcium sulphate, carbonate and phosphate; sodium sulphate, chloride, and

phosphate; ammonium chloride, sulphate and phosphate; potassium phosphate and magnesium sulphate. Oxidizing agents are often present as bromates, iodates, persulphates, nitrates, chlorates, perchlorates, and peroxides. Ferric salts are also now being used. These oxidizing agents are at times present in such small amounts that it is necessary to concentrate them before positive characteristic reactions can be obtained, or the improver contains such an abundance of cereal filler that the reactions are masked. Concentration of the mineral salts can be affected by means of a specific gravity separation using carbon tetrachloride. As a general rule, however, the tests can be applied without concentration.

In examining improvers, preliminary tests of moisture, ash, and protein give valuable information. pH determinations are also helpful in calculating hypothetical combinations. Ammonium nitrogen, calcium, magnesium, phosphate, sulphate, and chloride determinations are commonly required. The recommended methods of analysis are as follows:

General Methods

Moisture and Volatile Matter

Dry 5 gram samples in aluminum moisture dishes in a vacuum oven at a temperature of 100° C. to constant weight. When ammonium salts are present high results are obtained at this temperature and a lower temperature (50° C.) is suggested.

Ash

Ignite 5 gram samples in porcelain ignition capsules in an electric muffle furnace at a low red heat to constant weight. Reserve the ash for other determinations. It should be remembered that when phosphates are present low results are obtained.

Ammonium Salts

Qualitative Test. Place a few grams of the sample in a beaker, add a little water to make a thin paste and sufficient magnesium oxide to make the mixture alkaline. Heat on water bath. Ammonium salts may be detected by the odor of ammonia or by suspending a piece of moistened red litmus paper over the solution.

Qualitative Tests for the Nature of Ammonium Salts. The ammonium salt may usually be sublimed by heating the sample in a crucible over a burner and catching the sublimate on a cooled surface, such as the bottom of a procelain dish. This sublimed material may then be tested for ammonia, for chloride, or for sulphate, etc., by the usual methods. In this way, the nature of the ammonium salt may be determined.

Quantitative Determination. Place 1 to 5 gram samples (usually 2 grams) of the material to be tested into dry Kjeldahl flasks. Add 250 cc. of distilled water, a few pieces of zinc, a few cubic centimeters of mineral oil to prevent foaming, and sufficient magnesium oxide to make the solution alkaline. Immediately connect the flask to a distillation apparatus and distill off the ammonia into an excess of N/10 H_2SO_4. The excess of N/10 H_2SO_4 is titrated, using methyl red as the indicator. The distillation should be started slowly as the material may foam badly, increasing the heat when it is certain that the material will not foam over. The nitrogen is calculated as N and then as NH_4Cl or $(NH_4)_2SO_4$, etc., as indicated by the other tests.

Crude Protein

Crude protein may be determined by official A. O. A. C., or A. A. C. C. methods. Total nitrogen minus ammonium nitrogen equals protein nitrogen. Calculate the crude protein by using the proper factor, such as 6.25, 5.7, or 6.38, as indicated by the nature of the material as judged by its appearance, flavor, odor, or microscopical tests. If the material is wheat, use the factor 5.7; if milk, 6.38; but if it is a mixture of unknown source, use the factor 6.25.

Chlorides

Qualitative Test. Chlorine in yeast food is usually present in the form of soluble chlorides. It is generally satisfactory, therefore, to test the filtrate from a water suspension of the sample for the presence of chlorides by acidifying with nitric acid and adding silver nitrate. A white precipitate indicates chlorides.

Quantitative Determination. Weigh 0.5 to 5 grams of the sample (usually 1 gram) into 200 cc. beakers, add 100 cc. of distilled water, and stir well. Let stand for a short time, then filter. Wash the residue with water until free of chlorides. Collect the filtrate and washings in a 400 cc. beaker, add to them 5 cc. of concentrated nitric acid, and a known excess of N/10 $AgNO_3$ solution. Boil the solution for several minutes and then allow to stand on a water bath until the precipitate has completely coagulated. Cool the solution to room temperature, add 5 cc. of ferric ammonium sulphate indicator and titrate the excess of silver nitrate with N/10 KCNS. One cc. of N/10 $AgNO_3$ equals 0.003546 gram of Cl. Calculate the percentage of Cl and then the percentage of NH_4Cl, NaCl, $CaCl_2$ as indicated by other analyses.

Quantitative Methods for Determining the Mineral Content of the Ash in Yeast Foods and Flour Improvers [1]

Determination of Chlorides. Treat a previously weighed portion of ash with dilute nitric acid (1 + 9),[2] filter and wash if necessary, and determine chlorides by the procedure described above.

Determination of Calcium Magnesium, Potassium, and Sodium. Preparation of solution: Transfer the weighed ash from 5 gram samples of the material to be tested to a 400 cc. beaker and add from 10 to 20 cc. of concentrated HCl. Add the acid first to the crucible, and then to the beaker. Heat the strong acid solution for a short time, add 250 cc. of distilled water, and boil until no more of the solid material appears to go into solution. Filter into a 500 cc. volumetric flask and wash the residue thoroughly with hot water. If the residue after extraction is large, reignite and repeat the treatment with acid using a more dilute acid (1 + 2.5). Filter into the same 500 cc. volumetric flask as before. If the amount of insoluble matter still appears to be large this is probably silica. (To determine silica, ignite as for an ash determination, cool, and weigh.) Make the combined filtrates up to volume and use aliquots for the determinations to follow.

If the ashing period has been long and it is suspected that some of the ash may have been lost due to volatilization, the material under test may be charred for a short time at a low temperature and then treated with acid as outlined above. It is always advisable to ash a second time, and again treat with acid, when this method is used.

Determination of Calcium. Take 50 cc. of the filtrate (prepared as described in the paragraph above), the equivalent of 0.5 gram of sample, and add to it first, 5 cc. of a 10 per cent disodium hydrogen phosphate solution, then strong ammonium hydroxide until a permanent precipitate is obtained, and finally strong HCl drop by drop until the precipitate has dissolved. Boil for 2 to 3 minutes, cool, and mix with a considerable excess of 50 per cent ammonium acetate solution and 4 cc. of 80 per cent acetic acid.

As soon as the precipitate of aluminum phosphate, mixed with iron phosphate, has settled, collect on a filter, and wash thoroughly with hot water. Save the filtrate and washings for the determination of calcium.

If the amount of residue is large and it is desired to know the amount of iron and aluminum present, ignite the precipitate, cool, and weigh. Determine the ferric oxide and phosphorous pentoxide on this residue and subtract from the total weight of mixed phosphates to determine the alumina.

[1] In developing these methods access has been had to the Book of Methods of Analysis of the Association of Official Agricultural Chemists, 1925 edition, for which acknowledgment is hereby made.

[2] The expressions (1 + 9), (1 + 2), etc., used in connection with the name of a reagent mean that the volume of the strong reagent indicated by the first numeral has been mixed with the volume of water indicated by the second numeral.

Heat the combined filtrates and washings from the separation of iron and aluminum to 50° C., and add an excess of saturated ammonium oxalate solution. Let stand in a warm place until the precipitate settles (several hours or over-night), filter, and wash the precipitate with hot water. Save the filtrate and washings for the determination of magnesium. Dry the precipitate and ignite, first over a Bunsen burner, or in a muffle furnace: finishing the ignition over a blast lamp by heating to a constant weight. Cool in a dessicator, and weigh. From the CaO present calculate as the percentage of sulphate, phosphate, chloride, peroxide, etc.

Quantitative Determination of Magnesium. Evaporate the filtrate from the calcium determination to dryness on a water bath. Heat on a hot plate or sand bath to expel ammonia salts. Treat the residue with 20 to 25 cc. of hot water and 5 cc. of strong HCl; filter, and wash, confining the filtrate and washings to about 100 cc. Concentrate to 50 cc. and add 10 cc. of a 10 per cent disodium hydrogen phosphate solution, or enough of this reagent to precipitate all of the magnesium. Next, add gradually, strong ammonium hydroxide with constant stirring until the solution is distinctly alkaline. Add a few more cubic centimeters of the disodium hydrogen phosphate solution to insure complete precipitation of the magnesium. After 30 minutes, add gradually 10 cc. of strong ammonium hydroxide, cover the beaker to prevent the escape of ammonia, and let stand in the cold. Filter after 12 hours, wash the precipitate free from chlorides with diluted ammonium hydroxide (100 cc. strong ammonium hydroxide diluted to 1 liter), dry, ignite at a moderate heat in a muffle furnace or over a Bunsen burner until the carbon has been burned off, and then over a blast burner. Cool, weigh as magnesium pyrophosphate and calculate as magnesium oxide. Calculate as chloride, phosphate, sulphate, etc. as indicated by the analysis.

Determination of Potassium and Sodium. Place a 100 cc. aliquot, the equivalent of the ash from 1 gram of the original sample, into a 400 cc. beaker. Evaporate nearly to dryness to remove the excess of hydrochloric acid; dilute and heat to boiling. While still boiling, add a 10 per cent barium chloride solution as long as a precipitate forms. Next, add enough saturated barium hydroxide solution to make the liquid strongly alkaline. As soon as the precipitate has settled, filter and wash with hot water. Heat the filtrate to boiling; add sufficient ammonium carbonate solution (1 part of ammonium carbonate in five of dilute ammonium hydroxide solution (1 + 12)) to precipitate all of the barium; filter, and wash with hot water. Evaporate the filtrate to dryness and ignite the residue below redness to remove ammonium salts. Add to the residue a little water and a few drops of the am-

monium carbonate solution. Filter into a weighed platinum dish, evaporate, ignite below redness, and weigh the mixed potassium and sodium chlorides. To determine the amount of potassium in the mixed chlorides, digest the weighed residue with hot water, filter through a small filter and dilute the filtrate if necessary so that for each decigram of potassium oxide there will be at least 20 cc. of liquid. Acidify with a few drops of strong hydrochloric acid and add an excess of platinic chloride solution (containing the equivalent of 0.2 gram of metallic platinum in 10 cc.). Evaporate on a water bath to a thick paste, treat the residue repeatedly with 80 per cent alcohol, decanting through a weighed Gooch crucible or other form of filter; transfer the precipitate to the filter and wash thoroughly with 80 per cent alcohol. Dry for 30 minutes at 100° C. and weigh. If there is an appearance of foreign matter in the double salt, it should be washed 5 or 6 times with a solution prepared as follows: Dissolve 100 grams of ammonium chloride in 500 cc. of water, add 5 to 10 grams of pulverized potassium platinic chloride, and shake at intervals for 6 to 8 hours. Allow the mixture to settle overnight and filter. The residue may be used for the preparation of a fresh supply. After washing with this reagent, the precipitate is again washed thoroughly with 80 per cent alcohol and dried for 30 minutes at 100° C. Weigh and calculate the potassium so found to its equivalent as KCl and subtract this amount from the weight of the mixed chlorides to obtain the weight and percentage of NaCl. For the conversion of potassium platinic chloride to potassium chloride, use the factor 0.3067; to potassium sulphate, 0.35842; and to potassium oxide, 0.1937.

Determination of Phosphoric and Sulphuric Acid in Yeast Foods, etc. Determination of P_2O_5: Mix 5 grams of sample with about 5 cc. of magnesium nitrate solution. The magnesium nitrate solution is prepared as follows: Dissolve 160 grams MgO in dilute HNO_3 (1 + 1), add a little MgO in excess and dilute to 1 liter. Dry the magnesium nitrate paste, ignite, dissolve in dilute HCl (1 + 2.5), filter if necessary, and dilute to a definite volume, usually 250 or 500 cc. Take an aliquot of this solution, varying from 0.1 to 0.25 gram using the smaller amounts if the phosphate content of the sample is large, and a larger amount if the phosphate is small. Place in a 250 cc. beaker, add 5 to 10 cc. of strong nitric acid, then add strong ammonium hydroxide until but slightly acid and dilute to 75–100 cc. Heat to 45–50° C. and add an excess of molybdate solution which has been prepared as follows:

Molybdate Solution. Dissolve 100 grams of molybdic acid in dilute ammonium hydroxide (144 cc. of strong ammonium hydroxide and 271 cc. of water); pour this solution slowly and with constant stirring

into dilute nitric acid (489 cc. of strong nitric acid and 1148 cc. of water). Keep the mixture in a warm place for several days, or until a portion heated to 40° C. deposits no precipitate of ammonium phosphomolybdate. To 100 cc. of this solution add 5 cc. of strong nitric acid and filter immediately before using.

After adding the molybdate solution, allow the mixture to remain in the water bath at 45–50° C. with occasional stirring, for 30 minutes. Filter at once and wash with cold water until the filtrate from two fillings of the filter yields a pink color upon the addition of phenolphthalein and 1 drop of N/10 alkali. Transfer the precipitate and filter to the original beaker, dissolve the precipitate in a small excess of standard sodium or potassium hydroxide solution, add a few drops of phenolphthalein indicator and titrate the excess of alkali with standard hydrochloric or nitric acid. Calculate to P_2O_5 and then to calcium or magnesium phosphate, etc., as indicated by the analysis.

The standard acid and alkali solutions are made up as follows:

Standard Sodium or Potassium Hydroxide Solution. Dilute 323.81 cc. of N/1 alkali free from carbonates to one liter. One hundred cc. of the solution should neutralize 32.38 cc. of N/1 acid; 1 cc. is equivalent to 1 mg. of P_2O_5 (1 per cent of P_2O_5 on a basis of 0.1 gram of substance).

Standard Acid Solution. Prepare a hydrochloric or nitric acid solution corresponding in strength to the alkali solution, or to one-half of this strength and standardize by titration against that solution using phenolphthalein as indicator.

Determination of Sulphate. Boil 5 grams of the sample for 1½ hours with a mixture of 300 cc. of water and 15 cc. of strong hydrochloric acid. Filter, wash with hot water, and after cooling dilute the combined filtrate and washings to 500 cc. Heat 100 cc. of this solution to boiling, and add 10 per cent barium chloride solution slowly until no more precipitate is formed. Continue the boiling for about 5 minutes and allow to stand for 5 hours or longer in a warm place. Filter onto an ashless filter paper or a weighed Gooch crucible and wash with hot water until free from chlorides. Dry, ignite, and weigh as barium sulphate. Calculate to SO_3 or SO_4. In yeast foods most of the sulphate is usually combined with the calcium as calcium sulphate.

Detection and Determination of Oxidizing Agents

The oxidizing agents which will be considered are: Potassium bromate, potassium chlorate, potassium iodate, potassium nitrate, potassium perchlorate, benzoyl peroxide, calcium peroxide, ammonium persulphate, and potassium persulphate.

General Reactions which Indicate the Presence of Oxidizing Agents

There are several tests which give reactions with a large number of these compounds and these may be used to indicate the presence or absence of oxidizing agents. Prominent among them is the reaction with benzidine as well as the reaction with potassium iodide.

Reaction with Benzidine. Dissolve about 0.5 gram benzidine in hydrochloric acid (1 + 1) and then to small portions (10 to 25 cc.) of this reagent in test tubes or small beakers, drop in small portions of the yeast food from a spatula. If oxidizing agents are present, they will usually produce yellow or brownish streaks as the particles drop to the bottom of the container. This test will detect all of the oxidizing agents mentioned except perchlorate, nitrate, and benzoyl peroxide.

Reaction with Potassium Iodide. Place 50 grams of the sample in a 500 cc. Erlenmeyer flask and add 200 cc. of distilled water at room temperature. Shake well and allow to stand for about an hour with frequent shaking. Filter. To 5 cc. of filtrate add 5 cc. of 10 per cent potassium iodide solution and 5 cc. of sulphuric acid (1 + 10). Yellow or brown solutions will be obtained with all of the oxidizing agents mentioned except potassium nitrate, potassium chlorate, and benzoyl peroxide. Potassium perchlorate gives only a very weak reaction.

The reactions with benzidine and potassium iodide are easily carried out and are very useful in detecting the presence of most oxidizing agents. Special tests must be made to determine the presence of other oxidizing agents and these will be referred to later.

Detection and Determination of Individual Oxidizing Agents

Bromates and Chlorates

Qualitative Test for the Detection of Bromates and Chlorates.[3] To 5 cc. of an aqueous extract of the sample in a test tube add 5 cc. of aniline chloride in HCl (5 grams aniline chloride plus 100 cc. concentrated HCl, sp. gr. 1.18–1.19). In the presence of bromates, a deep blue color develops at once and fades rapidly. In the presence of chlorates the blue color develops slowly and a dark green precipitate is formed. Iodates also first give a blue solution which changes to a faint green. However, the presence of iodates may be confirmed by the thiosulphate test.

Qualitative Tests for the Detection of Bromates and Iodates—Optional Method. Shake 10 to 15 grams of the yeast food with about 50 cc. of bromoform in a separatory funnel. Let stand until the liquid is clear. Heavy bromate crystals will be found at the bottom of the liquid and can be drawn off, the bromoform decanted and the crystals washed

with ether. The crystals can be identified by dissolving in a small amount of water, acidifying slightly and adding about 1 cc. of carbon tetrachloride. Then add sodium bisulphite, a few crystals at a time, and a characteristic bromine yellow color will be found in the carbon tetrachloride layer. Potassium iodate also separates from the bromoform and may be drawn off in the same way as the potassium bromate but it gives a violet carbon tetrachloride layer instead of a yellow one and may be distinguished from the bromate in this way. This method gives excellent results. A word of caution is necessary. An excess of sodium bisulphite will destroy the bromine color. If an excess of bisulphite has been added, the color can be restored by adding chlorine water drop by drop.

Quantitative Determination of Potassium Bromate. Digest a 20 gram sample with 100 cc. of water at room temperature for 1 hour, stirring frequently. Filter. To 50 cc. of filtrate add 15 cc. of 50 per cent KI solution and 5 cc. of 10 per cent H_2SO_4. Neutralize with $NaHCO_3$, adding ether to break up the foam. Titrate with N/10 $Na_2S_2O_3$, using starch indicator. 1 cc. N/10 $Na_2S_2O_3$ = 0.0278 per cent $KBrO_3$.

Quantitative Determination of Potassium Chlorate.[3] Weigh 50 grams of the material to be tested into a 500 cc. Erlenmeyer flask and add 200 cc. of water. Shake well. Allow to stand for about 1 hour with frequent shaking and then filter. Measure 5 cc. portions of the filtrate into test tubes and add 20 cc. of a solution of aniline chloride (50 grams pure aniline chloride in 1000 cc. of HCl, 1.2 sp.gr.). To a series of test tubes containing known quantities of potassium chlorate diluted to 5 cc., add 20 cc. of the aniline chloride reagent, and after 25 minutes compare the color of the sample with standards of known concentration, and in this way determine the amount of chlorate present.

Iodates

Qualitative Test for the Detection of Iodates.[4] To 5 cc. of an aqueous extract of the sample in a test tube add 3 drops of starch solution (2 grams soluble starch to 100 cc. of water), 6 drops of H_2SO_4 (1 + 10) and then cover with 2 cc. of a 0.5 per cent solution $Na_2S_2O_3$. In the presence of iodates, a deep blue ring will be formed. This test is very good and gives satisfactory results.

Quantitative Determination of Iodates.[5] To 20 gram samples add 200 cc. of distilled water and mix well. Allow to stand 1 hour with

[3] Juan Fages Virgili. Detection and colorimetric determination of chlorates. Ann. Chim. Anal. Chim. Appl. **14**: 85–91.
[4] M. Dimitroff. Z. Anal. Chem. **52**: 452–453.
[5] W. W. Scott. Standard Methods of Chemical Analysis. 4th edition, p. 245, D. Van Nostrand Co., New York.

frequent shaking and filter. To 100 cc. of filtrate in a beaker add 1 to 2 grams KI and about 5 to 10 cc. of concentrated HCl. Allow to stand a few minutes and titrate with N/10 $Na_2S_2O_3$, using starch as indicator. 1 cc. N/10 $Na_2S_2O_3$ = 0.003567 gram KIO_3.

Nitrates

Qualitative Test for the Detection of Nitrates.[6] Add a few crystals of diphenylamine to about 5 cc. of concentrated H_2SO_4. Place a few hundredths of a gram of the dry sample to be tested on a watchglass, also place a few drops of the reagent on the watchglass. Tilt the watchglass and allow the reagent to mix with the powder. In the presence of nitrates, the mixture will be colored a deep blue.

Quantitative Determination of Potassium Nitrate. Weigh 40 gram samples of the yeast food and place them in 500 cc. Erlenmeyer flasks. Add 200 cc. of water, mix well and allow to stand for 1 hour with occasional shaking and then filter. Pipette 100 cc. of filtrate equivalent to 20.00 grams of sample into 500 cc. Kjeldahl flasks, add 150 cc. of distilled water, 60 cc. of 40 per cent NaOH, a few small pieces of pumice and about 5 cc. of white mineral oil. Distill to a low volume to remove ammonium salts. Dilute again to about 250 cc. and distill again to a low volume to insure the removal of all ammonium salts. In this second distillation it is advisable to catch the distillate in about 10 cc. of N/10 H_2SO_4 and then to titrate back with N/10 NaOH in order to determine whether all of the ammonium salts have been removed. If less than 0.5 cc. of N/10 H_2SO_4 has been neutralized by the liberated ammonia, it is safe to proceed with the analysis. If not, dilute and distill again to a low volume. Cool the residue in the Kjeldahl flask, add 3 grams of Devarda's alloy and connect with the usual Kjeldahl nitrogen distillation apparatus catching the liberated ammonia in 25 cc. of N/10 H_2SO_4. Allow the reaction to proceed in the cold for about $\frac{1}{2}$ hour and then apply heat very slowly at first and then more rapidly and continue the heating until about 150 cc. of distillate has been collected. Titrate the excess of N/10 H_2SO_4 with N/10 NaOH using methyl red as indicator. 1 cc. N/10 H_2SO_4 = 0.0101 gm. KNO_3.

Peroxides

Qualitative Test for the Detection of Benzoyl Peroxide. One or more thin and carefully smoothed layers of yeast food to be tested are spread on a glass plate. Pour over the layer or layers a solution containing 3 grams of benzidine, free base, dissolved in 100 cc. of 95 per cent

[6] Th. Sabalitschka and C. Schmidt. The detection of nitrates in plant and animal material. Ber. pharm. Ges. 33: 181–184.

ethyl alcohol. The benzidine solution should be heated in the water bath to about 120° F. before use. The presence of benzoyl peroxide is indicated by a few olive green to brownish specks which become more distinct and usually spread into diffusing streaks. This phenomenon may be observed with advantage from the back of the glass. Several tests should be made. Potassium persulphate produces a bluish green uniform color when tested in this way but no specks of olive green to brown color are formed.

Quantitative Determination of Benzoyl Peroxide.[7] To 10 gram samples in beakers add about 50 cc. of acetone. Stir and allow to stand for a few minutes to dissolve the benzoyl peroxide. Add 15 cc. of 10 per cent KI solution and 5 cc. of concentrated HCl and dilute with 50 cc. of water. Titrate immediately with N/10 $Na_2S_2O_3$.

When benzoyl peroxide has been mixed with and in intimate contact with oxidizable products, this method is not applicable.

Qualitative Test for the Detection of Calcium Peroxide.[8] Place 5 cc. of the following (50 cc. of a 10 per cent solution of KI plus 50 cc. of a 2 per cent soluble starch solution plus 200 cc. of milk) in a test tube. Add about 1 gram of original sample and shake. A blue color will be produced in the presence of calcium peroxide. Persulphates and iodates also give the reaction but they may be detected by other tests.

Quantitative Determination of Calcium Peroxide. To 20 grams of sample add 200 cc. of 1 per cent HCl. Digest for 1 hour, shaking frequently. Filter. To 100 cc. of filtrate add 5 cc. of 30 per cent KI solution and 5 cc. 1 + 1 (20 per cent) HCl. Let stand for one half hour and titrate with N/10 $Na_2S_2O_3$. 1 cc. N/10 $Na_2S_2O_3 = 0.0036035$ gm. CaO_2.

Perchlorates

Detection and Determination of Potassium Perchlorate.[9, 10] The method is based on a method developed for detecting and determining the presence of potassium perchlorate in saltpeter. A solution of methylene blue containing considerable zinc sulphate reacts with a nitrate solution containing perchlorate with the result that the color changes from dark blue to greenish blue. The necessary reagents are a zinc sulphate solution, sp. gr. 1.35 (about 50 per cent $ZnSO_4 . 7H_2O$); a methylene blue solution containing 1.6 grams of methylene blue in 5 liters of water, and a 20 per cent potassium nitrate solution. Twenty

[7] H. Gelissen and P. H. Hermans. Iodometric determination of benzoyl peroxide. Abstracted in Chem. Abst. **20**: 1611.

[8] Chas. D. Howard and Nathan Civen. Detection of hydrogen peroxide in beverages preserved with this compound. Ind. and Eng. Chem. **19**: 161.

[9] F. L. Hahn. Detection and determination of small quantities of perchlorate in Chili saltpeter and chlorate. Z. Angew. Chem. **39**: 451–454.

[10] Junck and Kupper. Comparison of colorimetric methods for the determination of perchlorate. Caliche **8**: 159–168.

cc. of the zinc sulphate solution are mixed with 5 cc. of methylene blue solution.

Quantitative Determination of Potassium Perchlorate. Weigh 20 gram samples of the yeast food into 250 cc. Erlenmeyer flasks and add from a pipette, 100 cc. of the 20 per cent potassium nitrate solution. Allow to stand for 2 hours with frequent vigorous agitation and then filter. Prepare standard solutions (about 25 cc. each) of the 20 per cent potassium nitrate containing 0.00, 0.05, 0.10, 0.15 . . . 0.60 per cent of potassium perchlorate. Into a series of test tubes of the same diameter and of such a size that they will fit into a color comparator, such as the La Motte Hydrogen Ion Determination Apparatus, measure by means of a pipette 5 cc. of the mixed zinc sulphate-methylene blue reagent. Measure 0.20 cc. of the filtrate from the sample and of the various standards into the tubes containing this reagent, mix and allow to stand for at least one hour, preferably 2 or 3 hours or longer. Place the tubes in the comparator and compare the colors. If the tubes containing the sample are lighter in color than the 0.00 of the standard, perchlorate may be present. Potassium and ammonium persulphates remove the blue color to a considerable extent, and calcium peroxide and benzoyl peroxide remove the color to a slight extent, but the presence of these compounds may be detected by other methods. The presence of 0.30 per cent of potassium perchlorate gives a light blue solution while the solution containing no perchlorate is deep blue, the difference being quite decided and easily detected. By matching the colors of the sample with those of the standards in a suitable comparator, such as the La Motte instrument, differences of 0.05 per cent or less of potassium perchlorate can easily be detected. If the tubes containing the samples are not distinguished, a frosted glass placed behind the samples, between the samples and the source of light, is of value in accentuating the differences in color.

Persulphates

Qualitative Tests for the Detection of Ammonium and Potassium Persulphates.[11] Extract, as described under the section "Reaction with Potassium Iodide." To 5 cc. of filtrate in a test tube add 2 cc. of a 0.5 per cent solution of benzidine in alcohol. Add the solution carefully, running it slowly on top of the sample so as to prevent mixing. Potassium and ammonium persulphates give a dense blue precipitate at the zone.

Determination of Ammonium and Potassium Persulphates in Yeast Foods.[12] Place 20 gram samples of the yeast food in 100 cc. glass

[11] A. Mannier. Characteristic reactions of perchlorates, periodates, persulphates, percarbonates, and perborates. Ann. Chim. Anal. Chim. Appl. **21**: 237–240.
[12] G. H. Mondolfo. Abstract in Chem. Abstr., 1928, 4083.

centrifuge tubes. Add 50 cc. of carbon tetrachloride and mix well with a glass rod. Remove the stirring rod and wash the adhering material into the tube with 25 cc. of carbon tetrachloride. Centrifuge for 5 minutes. Gently stir the upper compact layer of starch, etc., with a glass rod and then decant off the upper liquid and solid layers, being careful not to pour off any of the lower mineral layer. Add 25 cc. of carbon tetrachloride, stir and centrifuge again for 5 minutes. Pour off the upper layers as before and then place the residue in a vacuum oven at about 50° C. to remove the carbon tetrachloride. After the carbon tetrachloride has been removed, cool the residue and add 100 cc. of water by means of a pipette. Mix well. Allow to stand for about 30 minutes with occasional shaking and then filter. Place 50 cc. (10 gram sample) of the filtrate into a 250 cc. Erlenmeyer flask or a glass stoppered bottle of about the same size, add 15 cc. of 10 per cent KI and heat in a water bath at 60°–80° C. for 10 minutes. Cool and titrate with N/10 $Na_2S_2O_3$ using starch as indicator. 1 cc. N/10 $Na_2S_2O_3$ = 0.0135 gm. potassium persulphate. 1 cc. N/10 $Na_2S_2O_3$ = 0.0114 gm. ammonium persulphate.

Iron Compounds

Qualitative Tests for Detecting Ferric Iron. Potassium Ferrocyanide test: To about 5 cc. of an approximately 1 per cent solution of potassium ferrocyanide add about 1 gram of the yeast food and then acidify with a little concentrated hydrochloric acid. In the presence of ferric salts, the solution will be colored a deep blue.

Potassium Thiocyanate test: To about 5 cc. of an approximately 1 per cent solution of potassium thiocyanate add about 1 gram of the yeast food and then acidify with a little concentrated hydrochloric acid. In the presence of ferric salts, the solution will be colored a deep red.

Quantitative Determination of Added Ferric Compounds in Yeast Foods. Place 10 gram samples of yeast food in 250 cc. Erlenmeyer flasks and add 100 cc. of sulphuric acid (1 + 10) by means of a pipette. Shake well, allow to stand for one hour with frequent shaking and then filter through an acid washed filter paper (free from iron). Transfer a convenient aliquot of the filtrate (usually 10 cc.) to a 100 cc. Nessler tube. Add 10 cc. of 10 per cent KCNS solution and dilute to the mark. Place 10 cc. of the 10 per cent KCNS and 10 cc. of the 1–10 sulphuric acid in another 100 cc. Nessler tube and dilute to about 5 to 10 cc. below the mark. Add standard ferric iron solution to this tube until the color after diluting to a volume of 100 cc. is the same as that of the sample. The standard ferric iron solution, preferably a sul-

phate, should be prepared so that 1 cc. is equivalent to about 0.0001 gram Fe.

Yeast Food Fillers

The common organic diluents or fillers usually present in yeast foods or improvers are corn, potato, or wheat starch; malted barley or wheat; dry skim milk powder and sugar. These can readily be identified by the usual qualitative chemical, physical, or microscopical examinations. Microscopical comparisons with known specimens are very helpful in determining the nature of these fillers. Quantitative determinations of these various ingredients may be made by following accepted and approved methods.

Of the inorganic fillers, sodium chloride is by far the most common. In a few instances other inorganic fillers have been used and in such cases further tests and determinations may be desirable.

Acknowledgment

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A COMPARISON OF THE UTILITY OF FLOUR PROTEIN EXTRACTIONS IN DISTILLED WATER AND IN INORGANIC SALT SOLUTIONS

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Introduction

The question of the existence of a relationship between the water soluble protein of flour and baking strength has been mentioned in the literature by different workers in the field of cereal chemistry. However, no definite evidence appears to be available showing a correlation between these variables.

Rousseaux and Sirot (1913, 1918) concluded that the ratio of water soluble, to total protein, was not without significance in relation to baking strength, but it appears that part of their observations were based upon unsound flours. This fact may very well have influenced their results in so far as the variations of water soluble to total protein, which they encountered, are concerned. It has been stated by Bailey (1925) that various forms of unsoundness of wheat, such as sprouted, immature, and frosted kernels, cause an increased solubility of the nitrogenous constituents. The proportion of soluble protein also tends to increase with the degree of unsoundness, and unsoundness of grain may thus account for abnormally high percentages of water soluble forms of nitrogenous compounds in the flour milled therefrom.

Sharpe and Gortner (1923) extracted flours with distilled water for the purpose of removing the electrolytes, preliminary to determining the viscosity of acidulated flour-water suspensions. They extracted 18 grams of flour with distilled water, successively, twelve and sixteen times. Nitrogen determinations on the combined extracts indicated the removal of 9.35% of protein, and they concluded that practically all the gliadin had been extracted from the flour, as a subsequent analysis indicated that the sum of the albumin, globulin, and alcohol-soluble protein (gliadin) was 8.49%. The first extraction removed proportionately more protein than the others.

Tague (1925) found that gliadin dissolved in pure water, especially, if the gliadin was moist. He used an extraction period of 240 hours and a concentration of 1 gram of gliadin to 100 cc. of water. Nitrogen determinations on his water extracts showed the removal of 163.4 mgs. of the protein. Johnson (1926) while determining the effects of temperature, time of extraction, and manipulation, upon the quantity of electrolytes and proteins removed from flour and water suspensions,

found that substantial amounts of protein were extracted from flour by distilled water.

To determine whether protein solubility in water was correlated with baking strength, Harris (1931*b*) extracted a series of fifteen ground wheats with distilled water and correlated the extraction data with the loaf volumes obtained from the corresponding flours. The correlation obtained between non-extracted protein and loaf volume indicated a high relationship between these variables as the correlation constant for r was + .9696. The flours milled from these wheats were likewise extracted with distilled water and the data obtained correlated with the loaf volumes. The value obtained for the correlation constant in this case was $r = + .9455$. The significance of these correlations appeared to indicate the possibility of using distilled water in lieu of inorganic salt solutions for the purpose of determining protein solubility in relation to baking strength.

To ascertain whether the conclusions obtained from this series of wheats and flours would be confirmed by more extensive investigations, the following work was undertaken.

Experimental

Material and Methods Used

A series of forty-four experimentally milled 75% patent flours (see note 1), from wheat of the 1929 crop, was considered excellent material

TABLE I

Description of Wheat Samples and of Flour Milled Therefrom of the 1928 Saskatchewan Crop. Data on Basis of 13.5% Moisture

Laboratory number	Variety	Grade	Crude protein content of wheat	Crude protein content of flour	Ash content of flour	Bromated loaf volume
			P.ct.	P.ct.	P.ct.	cc.
488	Reward	No. 1 Manitoba Hard	16.6	15.6	0.43	865
877	Reward	No. 1 Manitoba Northern	16.1	15.4	.40	790
448	Marquis	No. 1 Manitoba Hard	15.0	14.1	.58	767
492	Reward	No. 1 Manitoba Hard	14.6	14.1	.40	747
494	Reward	No. 1 Manitoba Hard	14.3	13.8	not dtd.	725
473	Marquis	No. 1 Manitoba Northern	13.9	13.6	.44	687
465	Marquis	No. 1 Manitoba Northern	13.4	13.3	.52	687
486	Reward	No. 1 Manitoba Northern	12.7	12.3	.39	670
455	Marquis	No. 1 Manitoba Hard	12.4	12.1	.52	646
484	Marquis	No. 1 Manitoba Northern	11.8	11.1	.48	612
505	Kitchener	No. 1 Manitoba Hard	11.2	10.3	not dtd.	572
493	Garnet	No. 2 Manitoba Northern	10.5	10.0	.45	528
870	Marquis	No. 3 Manitoba Northern	9.3	8.7	.51	460

[1] The author's paper, "Relation between crude protein content and loaf volumes obtained by two different methods of baking." Cereal Chem. **7**: 557–570, Tables I and II, show data for 59 samples of wheat and flour, which include the 44 samples mentioned.

for the purposes of this study because (1), they were free from defect or unsoundness; (2), they were all of one variety (Marquis); and (3), they included a fairly wide range in baking strength and protein

TABLE II

SERIES 1. DESCRIPTION OF EXPERIMENTALLY MILLED FLOURS OF THE 1929 CROP. CRUDE PROTEIN, LOAF VOLUMES, AND EXTRACTION DATA ON BASIS OF 13.5% MOISTURE

Sample number	Crude protein in flour	Loaf volume		Total protein extracted by		Protein extracted by H_2O as per cent of flour	Protein non-extracted by H_2O as per cent of flour
		Basic	Improver	0.5N $MgSO_4$	H_2O		
	P.ct.	cc.	cc.	P.ct.	P.ct.		
25	16.0	529	730	15.2	19.9	3.18	12.82
14	15.2	525	650	15.2	21.2	3.22	11.98
13	14.7	520	680	16.0	21.7	3.19	11.51
43	14.7	505	644	15.7	16.9	2.48	12.22
44	14.5	535	650	16.6	19.6	2.84	11.66
35	14.2	545	630	15.8	18.1	2.57	11.63
65	13.9	505	625	15.0	20.1	2.79	11.11
29	13.8	520	670	16.9	19.6	2.70	11.10
31	13.6	525	615	17.7	20.9	2.84	10.76
36	13.4	534	605	15.4	20.1	2.69	10.71
16	13.1	500	550	16.9	27.1	3.55	9.55
9	12.9	475	560	16.0	22.5	2.90	10.00
22	12.9	500	525	15.2	21.0	2.71	10.19
39	12.8	530	552	17.4	20.9	2.68	10.12
24	12.7	502	610	17.1	25.3	3.22	9.48
4	12.6	455	555	15.8	21.6	2.72	9.88
26	12.6	472	620	16.6	21.0	2.64	9.96
41	12.5	495	558	17.3	20.5	2.56	9.94
42	12.4	515	580	17.8	22.1	2.74	9.66
40	12.2	505	530	17.1	20.4	2.49	9.71
8	12.1	490	500	15.7	23.0	2.78	9.32
1	12.0	540	535	17.9	27.7	3.32	8.68
6	12.0	470	500	17.9	22.5	2.70	9.30
5	11.9	465	515	16.1	23.5	2.80	9.10
7	11.7	440	475	17.6	20.7	2.42	9.28
2	11.5	475	515	17.7	26.5	3.05	8.45
21	11.5	446	490	18.8	18.9	2.17	9.33
32	11.5	490	540	17.8	23.1	2.66	8.84
10	11.3	465	465	19.3	21.4	2.42	8.88
20	11.3	463	560	19.0	24.8	2.80	8.50
19	11.2	443	500	18.3	19.8	2.22	8.98
62	11.2	470	490	17.5	17.8	1.99	9.21
3	11.0	450	440	17.3	23.0	2.53	8.47
37	11.0	490	540	19.9	27.9	3.07	7.93
38	10.8	507	516	17.6	18.7	2.02	8.78
64	10.8	475	510	16.3	19.1	2.06	8.74
34	10.7	475	510	19.1	20.4	2.18	8.52
33	10.6	505	525	19.7	23.2	2.46	8.14
15	10.5	450	455	18.4	24.7	2.59	7.91
23	10.4	487	520	19.8	26.1	2.71	7.69
28	10.4	500	505	16.7	21.1	2.19	8.21
17	10.1	445	470	19.4	24.9	2.51	7.59
63	9.1	435	440	19.3	19.0	1.73	7.37
61	9.0	445	410	20.5	18.0	1.62	7.38

content. No abnormality in respect to the behavior of these flours when extracted with distilled water would be expected from the character of the wheats. The flours were stored in tightly covered tins and no evidence of any change in their properties was apparent either from visual inspection or from a rebaking of a few of the samples preliminary to extraction with distilled water. This group of flours will be designated as Series 1, in this study.

A group of twenty millstream flours (see note 2) was also included in this study to ascertain the protein solubility, in distilled water, of flours of various baking strengths milled from the same wheat blend. In this connection it seemed desirable to determine the effect of the low quality streams, from the tail of the milling system, upon the relationship between total and water soluble protein, and water soluble protein and baking strength. This group of flours is designated as Series 2.

A third group of flours consisted of thirteen straight grade experimentally milled flours from the 1928 Saskatchewan wheat crop. These flours together with baking and analytical data were obtained from Dr. R. K. Larmour, University of Saskatchewan. These wheats were chosen for the purpose of obtaining information on flours of a longer extraction and from a crop of a different year. The very high relationship shown between the crude protein content of wheat and loaf volume of bread, $r = +.9908$, shows that these samples lie practically upon the regression line when a scatter diagram is constructed representing crude protein of wheat and loaf volume of bread. In Table I are given the grades assigned to these samples by a Government inspector, the variety of wheat, the crude protein contents of the wheats and the flours, the ash contents of the flours, and the loaf volumes (bromate method only) of the bread. It will be noticed that four varieties are included in this series and that the crude protein content ranges from 9.3% to 16.6%, a variation of 7.3%. Four of the thirteen samples were graded No. 1 Manitoba Hard; 7, No. 1 Manitoba Northern; 1, No. 2 Manitoba Northern, and 1, No. 3 Manitoba Northern. The flours in this series were treated with 0.5N solutions of the potassium halides as well as with distilled water in order to compare their relative protein solubilities in these media. This group of flours is designated as Series 3.

The flours of Series 1 were baked by a simple formula, called the basic formula, of flour, sugar, salt, yeast, and water; and also by a formula which included, in addition to the basic ingredients, 1% of diastatic malt and 0.001% of $KBrO_3$. If an increase of 10% or more in loaf volume was noted when the malt and bromate were added, the

[2] The author's paper, "A study of commercially milled flours dealing with protein and its relation to peptization and baking strength." Cereal Chem. 8: 113–133, Table IV, shows data for these flours.

flour was rebaked with 3% diastatic malt and 0.5% Arkady added to the basic ingredients. The volumes noted as "improver loaf volumes" are the values obtained with the use of these flour improvers, the malt and Arkady loaf volumes only being shown when the two improvers were used. In baking Series 2, the basic, the malt, and the Arkady methods were employed without the use of bromate, while in Series 3, only the loaf volumes obtained with bromate were considered in view of the fact that past work has shown the superior utility of improver baking methods as compared with the basic in bringing out the full potentialities of the flour.

The method employed for determining the extractable protein in these flours was according to the method developed by the author (1931). Briefly, this method calls for the weighing of 6 grams of flour into 250 cc. flasks (Erlenmeyer or Florence), adding 150 cc. of the liquid to be used as an extraction medium, and keeping the flour particles in suspension for one hour by frequent shaking. After allowing several hours for settling, the supernatent liquid is decanted in two 50 cc. portions and the extracted nitrogen determined by the Kjeldahl-Gunning procedure. No essential difference was noted between the behavior of the water and the inorganic salt solution extractions.

TABLE III

SERIES 2. DESCRIPTION OF MILLSTREAM FLOURS. CRUDE PROTEIN, LOAF VOLUME, AND COMPARATIVE EXTRACTION DATA ON BASIS OF 13.5% MOISTURE

Flour	Crude protein in flour	Loaf volume		Total protein extracted by			Protein extracted by H_2O as per cent of flour	Protein non-extracted by H_2O as per cent of flour
		Basic	Improver	$MgSO_4$	H_2O	H_2O-$MgSO_4$		
	P.ct.	cc.	cc.	P.ct.	P.ct.	P.ct.		
1st migglings	12.3	480	580	16.4	19.8	3.4	2.44	9.86
2d middlings	12.2	465	565	16.4	21.9	5.5	2.67	9.53
3d middlings	11.6	462	530	16.8	24.6	7.8	2.85	8.75
4th middlings	12.9	490	600	17.4	21.0	3.6	2.71	10.19
5th middlings	12.3	465	580	17.9	20.7	2.8	2.55	9.75
1st break	15.1	520	760	15.2	15.5	0.3	2.34	12.76
2d break	15.6	555	835	12.5	16.9	4.4	2.64	12.96
3d break	17.6	560	840	13.2	14.3	1.1	2.52	15.08
1st sizing	12.2	485	560	16.0	17.4	1.4	2.12	10.08
2d sizing	12.8	485	550	18.0	17.2	−0.8	2.20	10.60
Break cuts	15.6	542	780	15.1	17.6	2.5	2.74	12.86
6th middlings	12.7	480	555	19.8	16.7	−3.1	2.12	10.58
7th middlings	13.8	450	572	19.8	17.5	−2.3	2.41	11.39
8th middlings	14.3	400	435	28.8	21.2	−7.6	3.03	11.27
4th break	19.2	545	695	14.3	14.3	0.0	2.74	16.46
5th break	20.4	565	660	15.9	13.0	−2.9	2.65	17.75
1st tailings	15.8	500	645	16.8	14.1	−2.7	2.23	13.57
2d tailings	14.6	465	580	19.4	14.8	−4.6	2.16	12.44
Reel 2	18.3	530	610	16.6	13.3	−3.3	2.43	15.87
Reel 4	16.3	332	350	40.9	26.1	−14.8	4.25	12.05

The crude protein content of the flour, loaf volumes of the bread, and the water extraction data for the three series are given in Tables II, III, and IV, respectively. Table IV contains, in addition to the water data, peptization values obtained with four inorganic salt solutions, 0.5N KF, KCl, KBr, and KI. The detailed baking scores were omitted from these tables since loaf volume is subject to quantitative measurement. The data presented indicates a regular increase of loaf volume with crude and non-extracted protein, except for the low quality, millstream flours. The per cent of total protein extracted by water appears to be rather inconsistent in relation to protein content and loaf volume for Series 1. Several of the lower protein members of this group have relatively much lower percentages of the total protein removed by distilled water than one would expect from a knowledge of inorganic salt solution extractions. Comparing the H_2O and $MgSO_4$ extractions in this series, it is seen that water removes more of the total protein than $MgSO_4$ in all the flours except the two weakest. In Series 2, H_2O removed more protein than $MgSO_4$ in eleven of the twenty millstream flours, namely, the five best middlings flours, the first sizing flour, and the five best break flours. As the tail of the milling system is approached, the $MgSO_4$ values gain steadily, reaching a maximum excess over H_2O for Reel 4, which is the poorest flour in the group. From this it would appear that $MgSO_4$ acts first as a coagulating agent upon the protein of the best quality streams, but exerts an increasing peptizing influence as the flour quality progressively decreases. In Series 3, H_2O removes more protein than KF with the exception of two flours, where the difference was very slight. It would seem that KF exerts a coagulating effect upon the flour proteins largely similar to the action of $MgSO_4$ already noted.

A Hofmeister or lyrotropic series of anions is evident in proceeding from KF to KI, arranged in the order of increasing peptizing power $F < Cl < Br < I$. This arrangement corresponds to that noted by Gortner, Hoffman, and Sinclair (1929). Such a lyotropic series was also found by Johnson (1931). He extracted flour-water suspensions with 1.0N and 0.5N solutions of the sodium and potassium halides before determining the viscosities of the acidulated suspensions. He found these viscosities to be higher than the values obtained with water alone, and to increase in going from KF to KI. More protein was found to be removed from the flour in each case by the salt solutions than by water, with the exception of 0.5N KF, when the quantities removed were approximately equal. Johnson does not believe that the low results obtained by KF extractions can be attributed to inhibition of the action of phytase in rendering the phosphates soluble, and thus allowing them to depress the viscosity.

TABLE IV

SERIES 3. DESCRIPTION OF EXPERIMENTALLY MILLED FLOURS OF THE 1928 CROP (VARIETIES). CRUDE PROTEIN, LOAF VOLUME (BROMATE), AND EXTRACTION DATA ON BASIS OF 13.5% MOISTURE

No.	Crude protein of flour	Loaf volume	Percentage of total protein extracted by				
			H_2O	KF	KCl	KBr	KI
	P.ct.	cc.					
488	15.6	865	15.3	14.0	20.1	31.2	50.4
877	15.4	790	16.1	13.4	20.1	31.1	50.1
448	14.1	767	16.7	16.9	22.6	33.2	51.0
492	14.1	747	14.9	14.1	20.4	31.9	50.9
494	13.8	725	18.0	13.6	20.0	30.6	48.3
473	13.6	687	17.3	16.0	21.8	31.0	52.8
465	13.3	687	17.0	16.0	21.5	31.8	51.1
486	12.3	670	18.4	17.3	22.5	33.2	51.9
455	12.1	640	16.9	17.2	24.0	35.0	53.8
484	11.1	612	19.1	17.4	23.6	35.7	54.0
505	10.3	572	21.9	17.6	25.5	37.5	56.0
493	10.0	528	18.5	15.5	23.1	36.7	54.7
870	8.7	460	21.4	19.6	27.8	40.2	62.9
No.	Crude protein of flour	Loaf volume	Extracted protein as per cent of flour by				
			H_2O	KF	KCl	KBr	KI
	P.ct.	cc.					
488	15.6	865	2.39	2.18	3.14	4.87	7.87
877	15.4	790	2.48	2.06	3.11	4.79	7.73
448	14.1	767	2.35	2.38	3.19	4.68	7.20
492	14.1	747	2.00	1.99	2.87	4.51	7.18
494	13.8	725	2.48	1.87	2.77	4.23	6.66
473	13.6	687	2.35	2.18	2.97	4.22	7.18
465	13.3	687	2.26	2.13	2.86	4.24	6.80
486	12.3	670	2.26	2.13	2.77	4.09	6.38
455	12.1	640	2.04	2.08	2.91	4.24	6.51
484	11.1	612	2.12	1.93	2.62	3.96	6.00
505	10.3	572	2.26	1.81	2.63	3.86	5.77
493	10.0	528	1.85	1.55	2.31	3.67	5.47
870	8.7	460	1.86	1.70	2.42	3.50	5.47
No.	Crude protein of flour	Loaf volume	Protein non-extracted as per cent of flour by				
			H_2O	KF	KCl	KBr	KI
	P.ct.	cc.					
488	15.6	865	13.21	13.42	12.46	10.73	7.73
877	15.4	790	12.92	13.34	12.29	10.61	7.67
448	14.1	767	11.75	11.72	10.91	9.42	6.90
492	14.1	747	12.10	12.11	11.23	9.59	6.92
494	13.8	725	11.32	11.93	11.03	9.57	7.14
473	13.6	687	11.25	11.42	10.63	9.38	6.42
465	13.3	687	11.04	11.17	10.44	9.06	6.50
486	12.3	670	10.04	10.17	9.53	8.21	5.92
455	12.1	640	10.06	10.02	9.19	7.86	5.59
484	11.1	612	8.98	9.17	8.48	7.14	5.10
505	10.3	572	8.04	8.49	7.67	6.44	4.53
493	10.0	528	8.15	8.45	7.69	6.33	4.53
870	8.7	460	6.84	7.00	6.28	5.20	3.13

The data were subjected to a statistical study following the procedure employed by Geddes and Goulden (1930) and used by the author in former peptization studies. Total correlation coefficients were calculated between total protein and loaf volume, and extracted and non-extracted protein and loaf volume, for the three series of flours. The correlation coefficient r_{ae} is a standard measure for the relation between total crude protein content and loaf volume. The correlation between total crude protein and percentage of total protein peptized, r_{ad}, is stated by Geddes and Goulden to be a measure of the constancy of the peptized protein. They showed that the high negative values of r_{ad} obtained by them indicated that as the total protein increased the peptized protein did not increase relatively. These high negative relationships were not due to incomplete protein extraction.

The correlation coefficient between loaf volume and per cent of total protein peptized, r_{de}, is influenced partly by the relationship r_{ae}. Geddes and Goulden, therefore, used a method of analysis to measure the relative value of the peptized and non-peptized protein fraction. As it was impossible to hold a constant by partial correlations, these workers compared partial correlation coefficients, such as ${}_br_{ce}$ and ${}_cr_{be}$, for each salt solution and baking formula used. If c, the non-extracted fraction, is the more valuable, the value of ${}_br_{ce}$ should be higher than ${}_br_{be}$.

A second method of analysis was used to determine the practical significance of the total amount of information available from b and c in regard to the dependent variable e. This method employs the use of $R(bc)e$. An analysis of variance, founded upon the method of determining the significance of regression coefficients was also used by Geddes and Goulden in comparing the significance of the coefficients r_{ae} and $R(bc)e$, as these two coefficients cannot be compared directly due to the liability of they, themselves, being correlated.

TABLE V

STATISTICAL CONSTANTS COMPUTED FROM WATER EXTRACTION DATA OF SERIES 1

	Basic	Improver
r_{ae}	$+.6937 \pm .0527$	$+.9011 \pm .0191$
r_{ad}	$-.1883 \pm .0981$	
r_{be}	$+.5109 \pm .0752$	$+.5842 \pm .0670$
r_{bc}	$+.4324 \pm .0827$	
r_{ce}	$+.6531 \pm .0583$	$+.8715 \pm .0244$
r_{de}	$-.0403 \pm .1015$	$-.1465 \pm .0995$
${}_br_{ce}$	$+.5576 \pm .0701$	$+.8457 \pm .0290$
${}_cr_{be}$	$+.3346 \pm .0903$	$+.4690 \pm .0793$
$R(bc)e$	$+.7006 \pm .0518$	$+.9013 \pm .0191$

The results of this statistical study are shown in Tables V, VI, and VII. No significant relationship was found between per cent of

TABLE VI

STATISTICAL CONSTANTS COMPUTED FROM WATER EXTRACTION DATA OF SERIES 2

	12 Millstream flours		20 Millstream flours	
	Basic	Improver	Basic	Improver
r_{ae} =	+.9653±.0133	+.9631±.0141	+.4538±.1197	+.3928±.1275
r_{cd} =	−.7305±.0908		−.5922±.0979	
r_{bc} =	+.1184±.1920	+.2307±.1843	−.5612±.1033	−.4111±.1253
r_{be} =	+.0086±.1947		+.0250±.1507	
r_{ce} =	+.9569±.0144	+.9401±.0226	+.5676±.1022	+.4783±.1163
r_{de} =	−.7211±.0934	−.6388±.1152	−.7621±.0632	−.6336±.0902
${}_br_{ce}$ =	+.9627±.0142	+.9652±.0133	+.7029±.0763	+.5361±.1075
${}_cr_{be}$ =	+.3794±.1667	+.6529±.1117	−.6646±.0842	−.4547±.1196
$R(bc)e$ =	+.9631±.0141	+.9660±.0130	+.8083±.0521	+.6386±.0893

Note
a = total protein.
b = extracted protein.
c = non-extracted protein.
d = percentage of total protein extracted.
e = loaf volume.

total protein extracted by water and total protein, or between per cent total protein extracted and loaf volume in the first series of flours. Non-peptized protein is more highly correlated with loaf volume than peptized protein as shown by the correlation coefficients, ${}_br_{ce}$ and ${}_cr_{be}$. The improver method of baking is more valuable than the basic in this series of flours.

In the second series, a significant negative relationship is evident between per cent of total protein extracted and total protein, and between per cent of total protein extracted and loaf volume. The non-extracted protein is also more valuable than the extracted in this series. The improver method appears to increase the significance of the relation of the extracted protein to loaf volume for the twelve best quality millstream flours while leaving the other values unchanged. For the entire twenty flours the basic method seems more valuable.

In Series 3 the correlation r_{ae} is very high leaving only 3.6% of the total variability unaccounted for. It would seem that crude protein content would be an excellent means of forecasting loaf volume as would be expected in this series of flours. Per cent of total protein extracted is significantly related to total protein and to loaf volume. In general, the value ${}_br_{ce}$ decreases while the value ${}_cr_{be}$ increases, in going from H_2O to KI. The peptized protein, therefore, becomes more valuable as an increasing proportion of the good protein is

TABLE VII

STATISTICAL CONSTANTS COMPUTED FROM EXTRACTIONS AND BAKING DATA OF SERIES 3

H_2O	KF	KCl	KBr	KI
$r_{ae} = +.9817 \pm .0068$	$+.9817 \pm .0068$	$+.9817 \pm .0068$	$+.9817 \pm .0068$	$+.9817 \pm .0068$
$r_{ad} = -.8617 \pm .0482$	$-.7826 \pm .0725$	$-.8946 \pm .0374$	$-.9319 \pm .0246$	$-.8621 \pm .0480$
$r_{be} = +.1690 \pm .1817$	$+.2356 \pm .1767$	$+.8987 \pm .0360$	$+.9729 \pm .0100$	$+.9546 \pm .0166$
$r_{bc} = +.1566 \pm .1825$	$+.2138 \pm .1769$	$+.8689 \pm .0219$	$+.9394 \pm .0230$	$+.9344 \pm .0237$
$r_{ce} = +.9647 \pm .0130$	$+.9757 \pm .0090$	$+.9774 \pm .0083$	$+.9724 \pm .0102$	$+.9741 \pm .0096$
$r_{de} = -.8326 \pm .0574$	$-.7421 \pm .0841$	$-.8619 \pm .0481$	$-.8832 \pm .0411$	$-.8534 \pm .0508$
${}_br_{ce} = +.9637 \pm .0133$	$+.9746 \pm .0094$	$+.9051 \pm .0338$	$+.7386 \pm .0850$	$+.7738 \pm .0751$
${}_cr_{be} = +.0688 \pm .1862$	$+.1261 \pm .1841$	$+.4722 \pm .1454$	$+.7425 \pm .0839$	$+.5515 \pm .1302$
$R(bc)e = +.9648 \pm .0129$	$+.9761 \pm .0088$	$+.9826 \pm .0064$	$+.9879 \pm .0045$	$+.9821 \pm .0066$

Note a = total protein. b = peptized protein. c = non-peptized protein. d = per cent total protein. e = loaf volume.

TABLE VIII

COMPARISON BY ANALYSIS OF VARIANCE OF TOTAL AND MULTIPLE CORRELATION COEFFICIENTS

Correlation coefficient	Series 1 ($n = 44$)		Series 2 ($n = 12$)		Series 2 ($n = 20$)		Series 3 ($n = 13$) (Improver formula only)				
	Basic	Improver	Basic	Improver	Basic	Improver	H_2O	KF	KCl	KBr	KI
r_{ae}	.6937	.9011	.9653	.9631	.4538	.3928	.9817	.9817	.9817	.9817	.9817
$R(bc)e$	.7006	.9013	.9631	.9660	.8083	.6386	.9648	.9761	.9826	.9879	.9821
Z	.1280−	1.3650−	4.0623−	.1484−	1.5477+	.9929+	5.0165−	4.2728−	.3252−	.8171+	.7450−
Value of Z at 5% point	+.7085		+.8163		+.7466		+.8012				

removed. The simple correlation r_{bc} also shows the increasing importance of peptized protein in going from left to right in the table. This would appear to support the "optimum coagulation theory" of Kent-Jones. There is no tendency shown toward a reduction in the correlation coefficient between per cent of total protein extracted and loaf volume with increasing peptized protein. This is contrary to the findings of Geddes and Goulden (1930), who reported the correlation r_{de} much lower for KI than for $MgSO_4$.

In view of the fact that non-extracted or extracted protein, depending upon the salt used, has been shown to be in general more valuable than per cent of total protein extracted, the use of water as an extraction agent is permissible for the flours included in Series 1, notwithstanding the lack of relationship between extracted protein as per cent of total protein and loaf volume. Geddes and Goulden object to the use of per cent of total protein extracted as a means of forecasting loaf volume, due to non-linearity, of the relationship $(b/a)e$. It would accordingly seem that for the purpose of forecasting loaf volume by means of protein extraction in the three series of flours studied in this paper, distilled water is as useful as inorganic salt solutions.

An analysis of variance was applied to the total and multiple correlations r_{ae} and $R(bc)e$ for the purpose of determining whether any additional information was gained by a knowledge of peptized protein. Table VIII summarizes the tests carried out on all the pairs of r_{ae} and

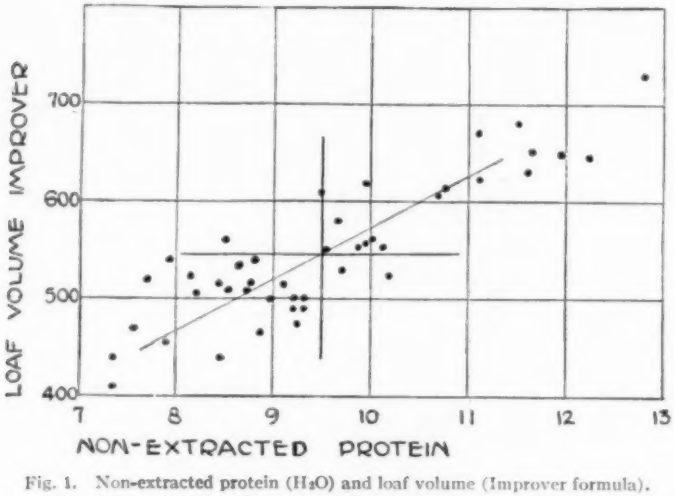

Fig. 1. Non-extracted protein (H_2O) and loaf volume (Improver formula). $n = 44$, $r = +0.8715 \pm 0.0244$

$R(bc)e$. A gain in accuracy is indicated by a plus sign and a loss by a minus sign. A significant gain over the simple correlation is indicated

by a Z value approximately the same or greater than the value at the 5% point. It appears from these values that only for the twenty millstreams in Series 2 is the utility of determining the peptized protein greater than that of the total protein. The basic value is the more significant. The lower relationship between total protein and loaf volume probably explains the comparative importance of the peptized protein here.

Figure 1 represents a scatter diagram of non-extracted protein in water medium and improver loaf volume for Series 1. A regular increase in non-peptized protein is shown with increasing loaf volume.

Summary

A series of forty-four experimentally milled 75% patent flours taken from the 1929 crop of Marquis wheat was baked by a simple basic formula of flour, water, sugar, salt, and yeast. These flours were then rebaked with the addition of 1% of diastatic malt and 0.001% of $KBrO_3$. Those flours showing an increase of 10% in volume as compared with the values obtained with the simple method were again baked with 3% diastatic malt and 0.5% Arkady. The loaf volumes obtained by the latter method were used in place of the bromate values in the list of improver loaf volumes.

A second series of 20 millstream flours was baked by the basic method, and also by the malt and Arkady method.

A third series of thirteen experimentally milled 95% extraction flours, of different varieties, from the 1928 Saskatchewan wheat crop was baked by a method including $KBrO_3$ in addition to the simple ingredients.

The flours of the three series were extracted by distilled water for one hour, the suspended particles allowed to settle and the extracted protein determined. The protein removed by similar treatment with 0.5N solutions of KF, KCl, KBr and KI was also determined for Series 3.

In Series 1, water extracted more protein than $MgSO_4$ in forty-two flours, and also more, in Series 2, for the eleven best quality millstreams flours. In Series 3, water extracted more protein than KF in eleven of the thirteen flours. Both $MgSO_4$ and KF apparently exerted a coagulating effect upon the water soluble flour proteins with the exception of low quality millstream flours.

The quantity of protein extracted in Series 3 was arranged in a Hofmeister series increasing with the increasing atomic weight of the peptizing agent, that is in going from KF to KI.

High positive correlations were obtained between crude protein of flour and loaf volume except for the twenty millstream flours.

High negative correlations were obtained between total crude protein of flour and percentage of total protein extracted and between loaf volume and percentage of total protein extracted, with one exception, in Series 1.

Partial correlations involving the use of extracted protein, non-extracted protein, and loaf volume showed very significant relations between non-extracted protein and loaf volume for water. The extracted protein showed no relation of any practical importance with loaf volume. Non-extracted protein became less significant in Series 3 in going from H_2O to KI, while the extracted protein became more valuable. This behavior may be explained by the "optimum coagulation theory" of Kent-Jones.

The simple or basic baking method gave inferior results for Series 1, was equal to the improver method for the best flours of Series 2, and superior for the twenty millstreams.

The use of the multiple correlations of extracted and non-extracted protein and the dependent variable, loaf volume, yielded little additional information than when total protein alone was considered.

Pure water would appear to be as useful as inorganic salt solutions for determining protein extractability with a view of forecasting baking strength.

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A STUDY OF METHODS FOR TESTING CAKE FLOUR

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(Read at the Convention, May 1931)

Keener competition has forced us to become millers of specialty flours. For years we have worked to improve wheat blends for hard wheat flour and we have studied different methods of treatment in order to improve the baking quality of such flour. We are now faced with much the same situation in the manufacture of cake, pie, and cookie flour. In other words, to market our soft wheat flour successfully, we must be just as careful in blending and milling our soft wheats as we have been in the manufacture of hard wheat flours.

It was recently my pleasure to visit the plant of one of the large pie manufacturers where I had a talk with his chemist. The first question he asked me was, "Is it possible for you to make for me an absolutely uniform pie flour?" He stated, "We have never been able to obtain a flour that was uniform for our purposes. The ash, protein, and gluten content, flour color, and even viscosity values may be the same, but at times different flours with all of these characteristics the same, will perform differently. It is our opinion that these changes are the direct result of a change in the wheat blend in the mill. We are not always able to detect these changes in our own laboratory, but they will show up at once under shop conditions."

I will not take the time to quote further the statements that this chemist made towards pie flour manufacturers. However, such statements do bring us face to face with a situation Cereal Chemists must recognize, namely, that we are being asked for specialty flours and the need for uniformity in this class of flours is essential.

Flour tests, which experience has shown, will give useful information to the bread baker, do not help very much with respect to supplying information indicating how soft wheat flours will handle in the cake, pie, or cookie shop. With us, it has been necessary to discard the tests applied to bread flour as a means of judging cake flour performance and to substitute in their place a more informative cake test. During the past few months we have devoted much time studying different cake formulae and test procedure in an effort to find one which would be satisfactory for our purposes, and the objective of this paper is to describe and discuss the work done to date.

Methods and Materials Used

In the making of this study the adaptability of four outstanding cake formulae were compared. As added interest, the usefulness of the A. A. C. C. bread baking test (basic procedure) was studied. The cake formulae studied were, (1) that of the Committee on the Testing of Biscuit and Cracker Flours of the A. A. C. C., (2) a hot water sponge cake formula, (3) a pound cake formula, and (4) a layer cake formula. Each formula studied as well as the baking procedure will be described under each series of tests. The flours used are described in Table I.

TABLE I

DESCRIPTION OF FLOURS USED IN COMPARATIVE TESTS

Flour number	1	2	3	4	5
Per cent protein	9.4	9.4	7.3	8.0	11.0
Per cent wet gluten	27.0	27.0	21.0	23.5	32.5
Gluten quality	Elastic-soft	Elastic-soft	Soft-short	Soft	Good-elastic
Viscosity [1]	44	44	18	26	80
Flour color [2]	2	4	1	3	5
Per cent ash	0.42	0.42	0.36	0.45	0.45
Blend	P. N. W.[3] Soft White	P. N. W. Soft white	Soft red winter	P. N. W. Soft red Soft white Club	Big bend Bluestem (Hard white)
Bleach	Novadel ½ oz. Agene 2 gm. Beta-Chlora 3/2 oz.	Novadel ½ oz. Agene 2 gm.	Novadel [4] Beta-Chlora [4]	Agene 2 gm. Novadel ½ oz.	Agene 5/2 gm.
Purpose recommended	Cake	Cake	Cake	General pastry	Cracker

[1] Degrees MacMichael from 20 grams of unwashed flour.
[2] Arranged in order of rank.
[3] Pacific Northwest.
[4] Bleached heavily with both Novadel and Beta Chlora.

The determination of usual chemical characteristics of the flour were made in the conventional way.

For viscosity measurements, the following procedure was used:

Twenty grams of flour, basis 15% moisture, and 100 cc. of water are mixed until smooth in a mortar and pestle. The viscosity of the suspension was first tested as is, using a No. 30 wire. A second reading was made after mixing 1 cc. of N/1 lactic acid into the suspension, a third reading after adding 2 more cc., a fourth reading after adding 2 additional cc., and a fifth reading after adding 2 more cc. The total amount of lactic acid used was 7 cc. The last two readings are generally the same. The maximum reading has been reported in Table I. The other readings can be plotted and are very informative, particularly for pastry and cookie flour.

Experimental

Use of the A. A. C. C. Bread Test in Evaluating Cake Flours

In this series of tests the main specifications of the A. A. C. C. tentative bread formula (Blish, 1928) were used. Mixing was accomplished by use of a Hobart three-quart mixer, using the cake paddle running at low speed. Sufficient flour and the other ingredients were used to make two duplicate test loaves. These were fermented as one dough, and scaled up, by dividing the dough when ready for the pans. This arrangement handles the dough conveniently and does away with hand mixing. On account of the extremely soft character of the several flours under test, the doughs were given only the first two punches prescribed in the tentative test; dough time was 2 hours and 35 minutes; proof 55 minutes. The data relative to the adaptability of the bread making test as a means of evaluating cake flour quality are shown in Table II. In Figure 1 are shown the external and internal appearances of the finished loaves.

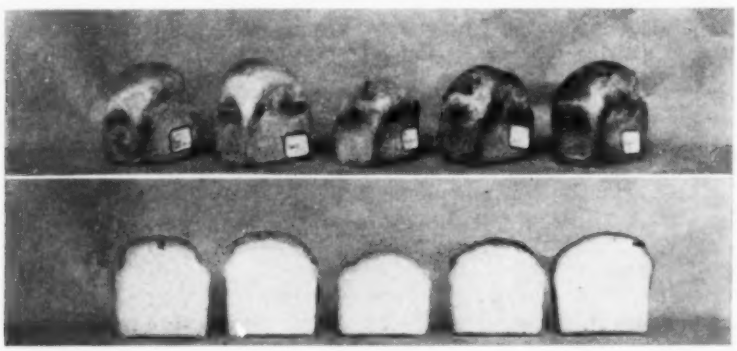

Fig. 1. External and internal appearance of bread made by A. A. C. C. standard baking test.

TABLE II

Bread Baking Tests and Cake Flour Quality

Flour number	1	2	3	4	5
Loaf volume cc.	540	560	440	500	560
Color of crumb	2 [1]	3	1	5	4
Texture of crumb	1 [1]	2	3	5	4

[1] Relative rank.

While these bread baking tests were informative with reference to the relative strength of the flours and were helpful in bringing out the color of the respective flour samples, there seems to be no clean-cut relation between bread making quality and cake making quality

except in extreme cases. A comparison of the bread making performance of the flours with their cake performances made later indicated that the best cake was made from the flour making the poorest bread, i.e., flour No. 3.

Results with the A. A. C. C. Tentative Cake Flour Test

The formula suggested by the Committee on Methods of Testing Cake and Biscuit Flours, Brooke (1929) was used. In these experiments sufficient shortening and sugar to make six cakes were creamed in a Hobart three-quart mixer at medium speed for ten minutes. The albumin, which had been dissolved in 50 cc. of water, was then added and the mass creamed for ten additional minutes. Sufficient of the creamed mass (125 grams) for each of five cakes was weighed out. The proper weight of each flour under test, 90 grams (to which had been previously added the salt and baking powder) was then added and stirred in with the water, 80 cc. All five cakes were baked in one-pound tins in the same oven, at the same time. By use of this procedure the flour was the only variable. Sufficient shortening and sugar mixture for six cakes was mixed en-mass so that it would not be necessary to use bowl scrappings. In testing the cake performance of flours of unknown character, a standard cake flour was baked up each time for comparative purposes.

The data relative to the tests made with the A. A. C. C. formula are shown in Table III, photographs of the cakes are shown in Figure 2, upper half.

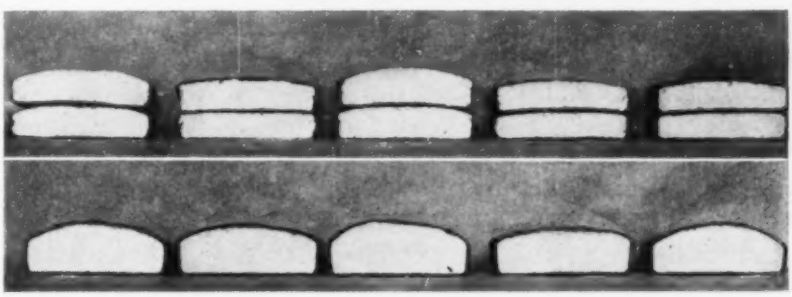

Fig. 2. Results of cake tests with A. A. C. C. cake formula (upper half) and sponge cake formula (lower half).

TABLE III

Results of Cake Baking Tests Using A. A. C. C. Formula

Flour number	1	2	3	4	5
Volume in cc. from 300 grams batter	740	730	830	710	730
Color of crumb	2[1]	3	1	4	5
Texture of crumb	3[1]	2	1	4	5

[1] Relative rank.

From the tests made with the A. A. C. C. cake formula it is to be concluded that this procedure is a satisfactory one for ordinary routine tests on cake flour. However, it appears to be deficient in one respect, namely, that by its use very good cakes can be made from cake flour which appears to be weak under bakeshop conditions. It is believed that a richer formula containing more sugar and shortening would produce a cake more nearly like that made under actual shop conditions.

Results With Hot-Water Sponge Cake Formula

The formula for a single cake as used in this series of tests is as follows:

I

Sugar	125 gms.	whipped light at 110° F.
Whole eggs	125 gms.	

II

Sugar	27 gms.
Crisco	20 gms.
Milk	48 cc.
Baking powder	2 gms.
Flour	165 gms.

The larger quantity of sugar and eggs should be beaten until real light over a water bath maintained at 110° F. The 27 grams of sugar, with the shortening, and milk, should also be warmed to 110° F. and then mixed into the eggs with the flour.

In these tests sufficient eggs and sugar for all five test cakes were measured into the 10 quart bowl of the Hobart mixer and beat until real light. Similarly, a solution was made of the sugar, shortening, and milk in sufficient quantity to make five cakes. This mixture was also brought to 110° F. The egg froth was divided into five parts and to each part was added sufficient of the previously prepared sugar, shortening, milk mass, to make one cake, along with the flour to which the baking powder had previously been added. Each test cake was scaled to 500 grams of batter into 1 pound tins. All cakes were baked at once at a temperature of 360° F. The results of the tests with the hot water sponge formula are recorded in Table IV. Photographs of the cakes by this formula are shown in Figure 2, lower half.

TABLE IV

Baking Properties of Cakes Baked by Hot-Water Sponge Formula

Flour number	1	2	3	4	5
Volume cc.	1250	1150	1260	1060	1150
Color of crumb	2 [1]	4	1	5	3
Texture of crumb	1 [1]	2	3	4	3

[1] Relative rank.

No large variation in cake making quality was evidenced by the flours when baked by the hot-water sponge formula. Texture difference were significant however. Cake flours Nos. 1 and 3 had very much finer texture of crumb than the other three flours tested. It is believed that this may be due to the fact that these two flours were the only ones bleached with Beta Chlora. This test is considered valuable for flour which is going to a shop where sponge cakes are made in large quantities, i.e., for bakers who require flour for sponge cake exclusively.

Results With Pound Cake Formula

The results of tests made with the pound cake formula are given in Table V, and photographs of the cakes are shown in Figure 3, upper half. The formula used in the pound cake series was as follows:

Sugar	150 gms.
Crisco	75 gms.
Whole eggs	75 gms.
Milk	75 cc.
Salt	2 gms.
Flour	150 gms.

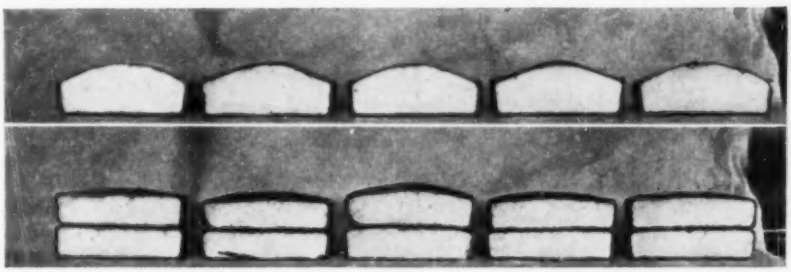

Fig. 3. Results of cake tests with usual pound cake formula (upper half) and layer cake formula (lower half).

The eggs, shortening, salt and sugar were creamed together. The sugar and shortening were first creamed for five minutes. The eggs were then slowly added in small portions consuming five minutes in the operation. This egg-shortening and sugar mass was then creamed for 10 additional minutes. Total time of mixing was 20 minutes. Sufficient of the mass was mixed up at one time to make five cakes. The Hobart mixer was used with the cake paddle operating at medium speed. The creamed mass was divided in five parts, and milk and flour in sufficient amount to make one cake were added. The batter was scaled at 500 grams. The cakes were baked in one-pound bread tins at 360° F.

TABLE V

RESULTS OF TESTS USING THE POUND CAKE FORMULA

Flour number	1	2	3	4	5
Volume cc.	1354	1280	1200	1280	1280
Texture of crumb	5[1]	1	4	3	2
Color of crumb	3[1]	2	1	4	5

[1] Relative score.

Differences in volume are not great by this method. The weakest flour showed the smallest volume. The results of the tests by the pound cake formula were much like the tests obtained by use of the A. A. C. C. bread test. While this test is not recommended for judging cake flour, it has proven very helpful in testing other ingredients in the laboratory. This formula shows differences in egg quality readily and tests made with hydrogenated shortenings have shown differences in cake volume by this formula by more than 500 cc.

RESULTS USING LAYER CAKE FORMULA

The procedure with regard to creaming the eggs, shortening, and sugar in this series of layer cake tests was the same as used in the pound cake series. The formula for one cake is as follows:

Sugar	120 gms.
Crisco	60 gms.
Whole eggs	60 gms.
Milk	75 cc.
Salt	2 gms.
Baking powder	6 gms.
Flour	120 gms.

The salt and baking powder were sifted into the flour samples. Sufficient of the creamed mass was made up for all five cakes, and later divided into five parts and the flour and milk stirred into each cake. The batter was scaled at 400 grams and the cake was baked in one-pound bread tins at 380° F. The results of the tests with the layer cake formula are given in Table VI and photographs of the cakes are shown in Figure 3, lower half.

TABLE VI

RESULTS OF BAKING TESTS USING LAYER CAKE FORMULA

Flour number	1	2	3	4	5
Volume cc.	1000	990	1110	990	980
Texture of crumb	2[1]	4	1	5	3
Color of crumb	2[1]	4	1	4	5

[1] Relative ranks.

The differences brought out by the use of the layer cake formula are much the same as obtained by use of the A. A. C. C. cake formula. The test is not so good with respect to color, because of the use of egg yolks. A white cake can be obtained by using only the whites of the eggs.

This formula is liked because it seems to produce a cake which will show all the different points. The cake is of good flavor, and is suitable to take out to the trade. The layer cake formula is somewhat richer than the A. A. C. C. formula, inasmuch as the sugar and shortening are about in the same ratio most often found in the bake shop.

Conclusions

A cake test is better than a bread test for evaluating cake flour.

The A. A. C. C. cake test is considered excellent for routine laboratory work. The A. A. C. C. test has the disadvantage of giving high tests on weak flours.

The layer cake formula was found to be a very desirable formula.

Viscosity tests do not seem to help in evaluating cake flour.

The fact that a flour shows up well in one formula is not a guarantee that it will make an equally as good a cake with another formula.

Short patent, finely ground flours produced the best cakes.

A granulation test should prove helpful in valuing cake flour.

The cake making properties of flour seems to be benefited by the use of Beta Chlora as a bleaching agent.

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RANCIDITY[1]

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Deterioration of fats through rancidity development has been a long recognized fact, but until recently there has been meager information available regarding the processes involved. This has been due in part, at least, to the conflicting views held regarding the nature and cause of rancidity.

A survey of the literature will reveal three general types of fat deterioration, each considered as rancidity, although the processes of decomposition and end products obtained are very different These three types of deterioration may be termed oxidative, hydrolytic, and ketonic rancidity. The development of oxidative rancidity is considered to be due to the addition of molecular oxygen to unsaturated glycerides with the formation of peroxide or oxide like compounds, which subsequently decompose into aldehydes, ketones, and fatty acids. This type of reaction may occur in all edible fats, since they all contain unsaturated glycerides, and is, for this reason, a very important type of fat spoilage. In the development of hydrolytic rancidity there is an hydrolysis of the glycerides with the liberation of free fatty acids as end products. This type of rancidity is of special importance in the spoilage of dairy products, due to the liberation of butyric acid with its characteristic odor and taste. The liberation of small amounts of the other fatty acids does not appreciably affect the odor and taste of a fat. Fats containing nitrogenous impurities, such as coconut oil, may undergo ketonic rancidity. This is effected through the action of certain molds on lower members of the saturated fatty acid series with the production of methyl ketones as end products.

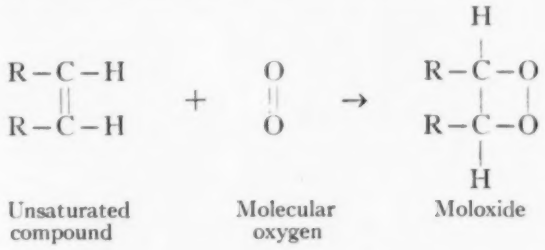
Rancidity development in edible shortening agents and in baked goods is due ordinarily to oxidative deterioration. It is for this reason that this paper has been confined solely to oxidative rancidity.

Theories Regarding the Development of Oxidative Rancidity

All the theories on the mechanism of oxidative rancidity have had their origin in the observation of Engler and Weissberg (1904), that molecular oxygen can attack "double bond" or "unsaturated" linkages, in a manner similar to that of ozone. The resulting compound of this autoxidation process contains a peroxide group and is

[1] Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station as technical paper 529.

referred to as a moloxide. Staudinger (1925) defines "moloxides" as peroxide compounds which have as yet not been isolated and consequently are of unknown composition and structure.



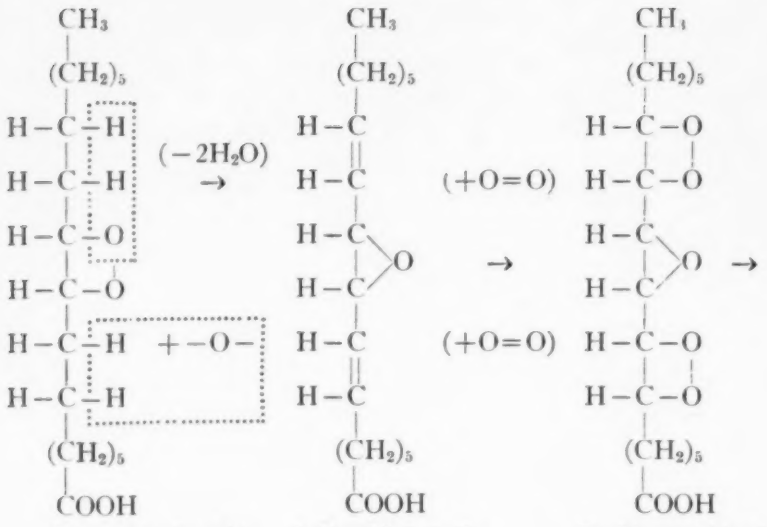
This moloxide readily breaks up into aldehyde and other decomposition products. Engler and Weissberg indicated that the unsaturated fatty acids were subject to this type of autoxidation.

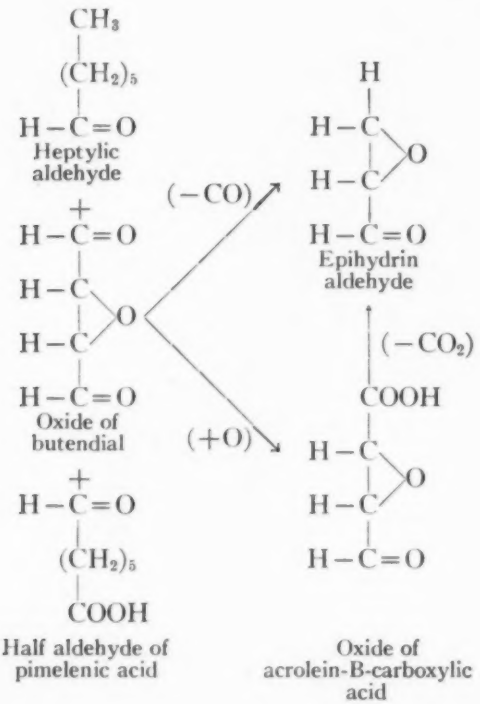
This conception of autoxidation was then applied to fats. Vintelesco and Popesco (1915) used a peroxide test to detect rancidity in fats. Kerr and Sorber (1923) also used peroxide tests for the detection of rancidity. They suggested that these peroxide compounds, being relatively unstable, would act as extremely good oxygen carriers to favor the oxidation of unchanged glycerides with which they were in contact.

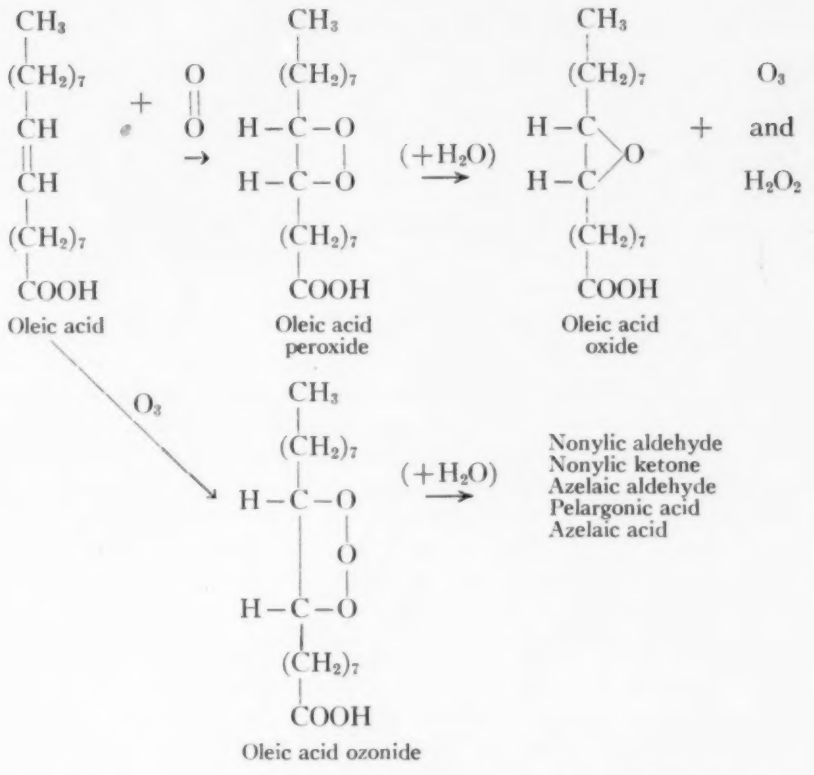
Powick (1923) used the theory of Engler and Weissberg to account for the products present in rancid oleic acid. He found the rancid odor and taste due to heptylic and nonylic aldehydes while the Kreis test for rancidity depended upon the presence of epihydrin aldehyde. Powick advanced the following hypothetical mechanism to account for these substances. Oleic acid adds molecular oxygen to form oleic acid peroxide, which subsequently loses water to form an oxide. This oxide, after the addition of two molecules of oxygen, splits up into heptylic aldehyde and other compounds, one of which can form epihydrin aldehyde through the loss of carbon monoxide.

The theory postulated by Tscherich (1925) differs somewhat from that of Powick's in that the peroxide formed is decomposed by water giving oxide, hydrogen peroxide, and ozone. The ozone is the reactive constituent and reacts with other unsaturated acids present forming ozonides, which in the presence of water are easily split into aldehydes and acids. The hydrogen peroxide may act as a bleaching agent in the fat.

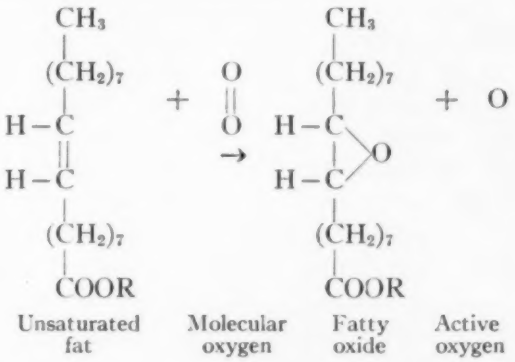
A still somewhat different theory was postulated by Browne (1925). His idea was that a molecule of oxygen attacks the double bond, forming a fatty oxide and liberating an atom of active oxygen for every atom of oxygen absorbed. The active oxygen liberated,

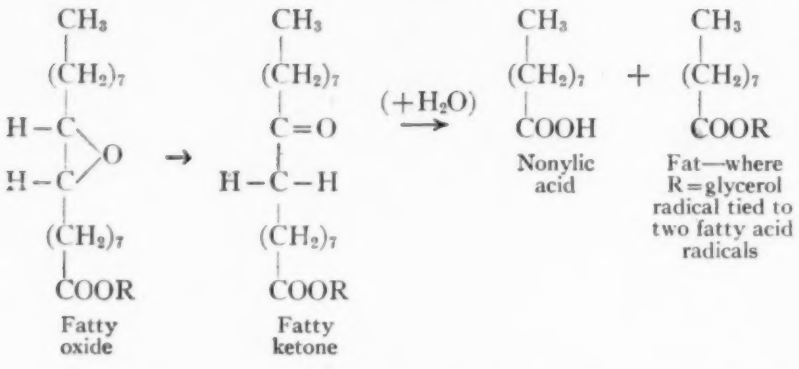

Oleic acid peroxide
Peroxide oxygen
Oxide of 7–8, 9–10, 11–12 linolenic acid
Double peroxide of oxide of 7–8, 9–10, 11–12 linolenic acid


Heptylic aldehyde
Oxide of butendial
Half aldehyde of pimelenic acid
Epihydrin aldehyde
Oxide of acrolein-B-carboxylic acid

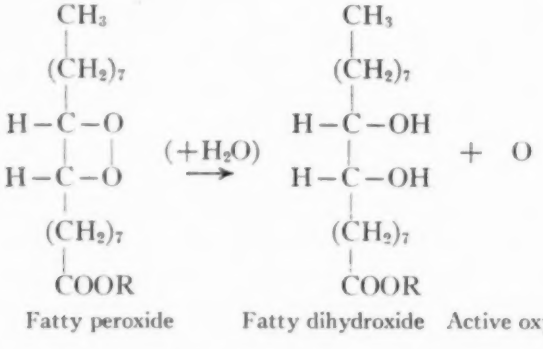


immediately acts upon the glycerides with which it is in contact and causes them to break up into free fatty acids, aldehydes, carbon dioxide, water and other decomposition products. Some of the active oxygen could also unite with the water present to form hydrogen peroxide. The fatty oxide, after rearranging to a ketone form might then decompose in the presence of water to a lower fatty acid. These reactions would proceed as indicated:





Browne also indicated in the event that a peroxide compound was formed, as postulated by Engler and Weissberg (1904) or Tscherich (1925), the reaction might go differently than these investigators stated. Such a peroxide might form a di-hydroxide with the liberation of active oxygen as indicated.



The various theories regarding the development of oxidative rancidity all agree that molecular oxygen attacks the unsaturated glycerides with the formation of a peroxide or oxide group at that point. These loosely combined oxygen compounds either decompose spontaneously into, or react with water to form aldehydes, ketones, fatty acids, active oxygen, ozone, and hydrogen peroxide. The liberation of active oxygen and ozone may cause the decomposition of glycerides, which would not ordinarily be attacked by molecular oxygen. No free glycerol can be detected in rancid fats, neither does the acidity of the fat need to increase with rancidity, consequently we assume that oxidation of the glycerides may take place independently of hydrolysis, a fact which is substantiated by the work of Nicolet and Liddle (1916).

Tests for Oxidative Rancidity

The first tests for rancidity were physical ones, depending upon the senses of taste and smell, and these are still the only tests upon which there is universal agreement by all investigators. Such tests, however, are apt to be influenced by personal opinion, and are hard to express in degree of rancidity. As a result, considerable research has been conducted to determine physical and chemical means for the detection of rancidity and for the numerical evaluation of its intensity.

The tests most commonly used have been color tests, of which the most important have been the peroxide, aldehyde, and Kreis tests. Peroxide tests were used by Vintelesco and Popesco (1915), Kerr and Sorber (1923), and Powick (1923) for the detection of rancidity. These tests should be particularly applicable since peroxide compounds are the first products postulated in rancidity development. While the peroxide tests always react with a rancid fat. the intense color formed makes the evaluation of the degree of rancidity rather difficult.

Rancid fats also respond to the various aldehyde tests recommended by Schmid (1899) such as: (1) ammoniacal silver solution; (2) *m*-phenylene-diamine; and (3) 0.5% fuchsin sulphurous acid. Of these, only the fuchsin sulphurous acid test has been further recommended by Von Fellenberg (1924), who claimed that this test gave better results than the Kreis test. An extensive and complete comparison of the Kreis and Von Fellenberg tests was made by Pritzker and Jungkunz (1926) who concluded that the Von Fellenberg test had no advantage over the Kreis test. Just recently Schibsted (1931) has refined the test so that it is more sensitive and also permits an evaluation of the degree of rancidity. He expresses the content of aldehydes in a fat or oil in arbitrary color units obtained per unit of fat and suggests that this specific color value be called the "Fat Aldehyde Value." Schibsted found the improved reagent to be over 20 times as sensitive as any of the other reagents.

The most widely used test for the detection of oxidative rancidity is the Kreis test originated by Kreis (1902). The test consists in thoroughly mixing 1 cc. of a fat or oil with 1 cc. of concentrated HCl, and then adding 1 cc. of a 1% solution of phloroglucinol in ether. If after thorough mixing, the separated acid layer has acquired a red or pink coloration, the oil is rancid, and the intensity of the color is a rough indication of the degree of rancidity.

Kerr (1918) modified the test somewhat so that it served as a means of determining the degree of rancidity. He shook up 10 cc. of oil or fat with 10 cc. of concentrated HCl and 10 cc. of a 0.1%

solution of phloroglucinol in ether. If a red color developed in the acid layer, he diluted the original oil or fat with kerosene until a dilution was reached where no color reaction was obtained. The amount of dilution required was then an index of the degree of rancidity.

The main criticism of the Kreis test has been the inconsistent results obtained when the test has been applied to cottonseed oil. It was shown by Smith (1920) that naturally occurring substances were often present in cottonseed oil and would react to the Kreis test. These substances, however, were not glycerides and were not formed by the decomposition of glycerides, since the color produced in the reaction developed more slowly and was of a more purplish red shade than that produced by a rancid oil. Powick (1923) corroborated these results by a spectrophotometric study of the Kreis test, and demonstrated that by such an examination, it could be determined definitely whether the color was due to a rancid condition of the oil or to some other substance present.

In making a Kreis test, it is very essential to adhere to a rigid procedure, especially, if an evaluation of the color intensity is desired. If, for example, the order of adding the reagents is changed, colored solutions may be obtained of different intensities for the same amount of rancid fat and reagents. It is also necessary to insure that the reagents used are pure. Powick (1928) found that hydrochloric acid may contain nitrosyl chloride as an impurity, and as such, would give an intense red color with phloroglucinol but no color could be obtained when added to a non-rancid or rancid fat and phloroglucinol. Ether used in preparing the phloroglucinol solution or for extracting fat to be used in the test, frequently contains a peroxide contaminant which gives a reddish brown color in the Kreis test. Under such conditions, ether must be purified before it can be used. It is obviously necessary to always insure that a "blank" test, using only hydrochloric acid and phloroglucinol solution, gives no reddish color.

The intensity of the color produced in the Kreis test was found by Kerr (1918) to be roughly proportional to the degree of rancidity, while Holm and Greenbank (1923) found that the intensity of the color produced was directly proportional to the amount of oxygen absorbed by the fat. Since Kerr had based his results upon the dilution of a fat until it gave no color with the Kreis reagents, and Holm and Greenbank had matched the colored solutions obtained from the Kreis test with a set of standard color tubes, a somewhat more refined study of the test was undertaken with the aid of a spectrophotometer. It was desired to determine just how large a variation in intensity of the Kreis test might be expected between

samples of the same type of shortenings with equivalent oxygen absorptions. In the event that the difference between individual samples was not very great, such a study would be of considerable value in developing a method for determining the oxygen absorption (or degree of rancidity) of any sample of shortening.

The oxidation of the fat to definite oxygen absorptions was accomplished in a modification of the Holm and Greenbank (1923a) gas-tight stirring apparatus. The fat was oxidized at 95° C., samples being withdrawn at definite oxygen absorptions. Two grams of the oxidized fat were weighted into a small vial, and treated with 2 cc. of concentrated HCl and 2 cc. of a 1% solution of phloroglucinol in ether. After complete mixing, the contents were allowed to separate into layers, or centrifuged if necessary, to effect a separation. One cc. of the colored solution was then diluted to a 10 cc. volume with alcohol and filtered. A 2 cm. spectrophotometer tube was filled with this solution and examined in a Keuffel and Esser direct-reading spectrophotometer, and the percentages of light determined which were transmitted through the tube at the various wave lengths.

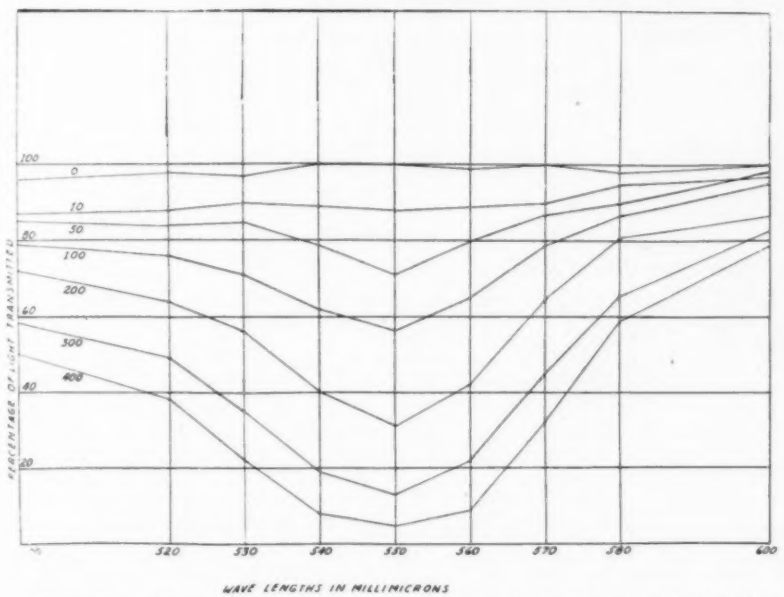


Fig. 1. Percentage of light transmitted at various wave lengths by the colored solution produced by the Kreis reagents on a prime steam lard with 0, 10, 50, 100, 200, 300, and 400 cc. of oxygen absorbed per 100 grams of lard.

It was found that the greatest absorption of light by the solutions occurred at the wave lengths 530, 540, 550, and 560 and 570 millimicrons. Figure 1 illustrates graphically the percentage of light transmitted at the various wave lengths by the colored solutions

obtained by the Kreis test on a prime steam lard sample with definite oxygen absorptions. Not only did the greatest absorption of light occur at these five forementioned wave lengths, but also, the percentages of light transmitted could be more easily and accurately determined at these wave lengths than at any others. For these reasons, it was deemed advisable to take the mean of these five readings as indicating the percentage of light transmitted by the colored solutions, representing a definite oxygen absorption by the fat. Since the percentage of light transmitted bears an inverse relationship to the percentage of light absorbed, or stated a little differently, to the color intensity of the solution, the reciprocal of the percentage of light transmitted indicates more clearly the relationship of oxygen absorption to the color intensity of the Kreis test on fats and oils. It was found that when the log of the reciprocal of the percentage of light transmitted was plotted against the amount of oxygen absorbed by the fat or oil, that practically a straight line relationship existed.

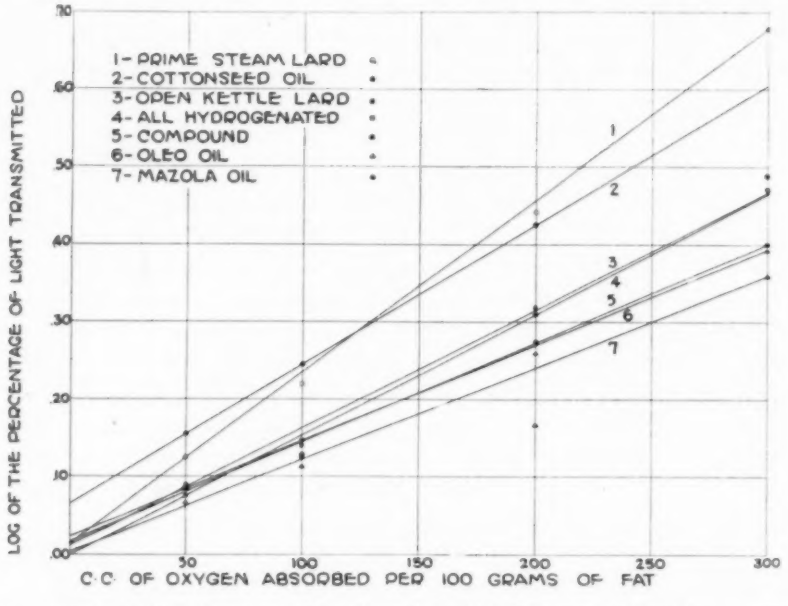


Fig. 2. Intensity of the Kreis test on various shortenings with definite oxygen absorptions. Intensity indicated by the log of the reciprocal of the percentage of light transmitted.

The most striking result indicated in the study (Figure 2) is that with the exception of cottonseed oil and prime steam lard, all shortenings with identical oxygen absorptions, gave Kreis tests of similar intensities. Cottonseed oil showed an initial absorption of light by the unoxidized oil, and if this is deducted, the results for the cottonseed oil approximate those for the all-hydrogenated shortenings. It would

appear from these results that the quantitative determination of the degree of oxidative rancidity could be fairly accurately determined for all shortenings especially at the lower oxygen absorptions by a spectrophotometric study of the intensity of the Kreis test. It should be emphasized that this work has only been of a preliminary nature and considerably more needs to be done before any definite conclusions can be drawn. These results were taken from some unpublished data obtained while at the University of Minnesota.

Methods for Studying the Susceptibility of Fats to Autoxidation

Various methods have been used to study the susceptibility of fats and oils to oxidative rancidity. Two of these methods, the controlled oxidation method of Greenbank and Holm (1925) and the methylene blue reduction method appear to yield the most promising results.

The controlled oxidation method consists briefly in weighing a definite amount of fat or oil into a flask, replacing the air within the flask by oxygen, and keeping the whole at a constant temperature in a carefully controlled thermostat. The time required for the sample to start reacting with oxygen, as registered by a fall in pressure within the system, is carefully noted and the amount of oxygen absorbed for definite intervals of time thereafter is determined.

The length of time a fat or oil is in intimate contact with oxygen before any consistent and measurable absorption takes place is taken as the induction period. The length of this period is then an indication of the ease with which a fat or oil will oxidize, or, is a measure of susceptibility to oxidative rancidity. This has been very clearly demonstrated by the results of Greenbank and Holm (1924) on butterfat.

The rate of methylene blue reduction in a fat under constant conditions has also been used as a means of determining susceptibility to oxidative rancidity. Greenbank and Holm (1930) have recently devised a method whereby the reduction of methylene blue by a fat is catalyzed by light and the point at which a definite reduction has taken place is indicated by a photoelectric cell. An appreciable decrease in the length of time of the experiment is obtained by this method.

A somewhat different procedure for performing the methylene blue test was used by Davies (1928). By this test, the fat to be examined is mixed with methylene blue and emulsified in skim-milk. This mixture is incubated at 37° C. and the blue color reduced by the milk reductase. After all the color has disappeared, the tubes are shaken vigorously for 15 seconds and allowed to stand 2 minutes.

The amount of restoration in color at the end of this period is a measure of the oxidation capacities of the fat or the ability to make use of the dissolved oxygen.

Factors Influencing Oxidative Rancidity

Controlled oxidation methods and methylene blue reduction tests have made possible the rapid and accurate determination of the effect of various factors upon oxidative rancidity. Thus, Greenbank and Holm (1924) found that fatty acids decreased the length of induction period of oxygen absorption of fats, the effect being greatest with the longer chain acids. Moisture, on the other hand, was found to increase the length of induction period of oxygen absorption, indicating a retarding effect upon oxidative rancidity. This is contrary to the role of moisture in hydrolytic rancidity, where it is essential for the hydrolysis of the glycerides. Fine and Olsen (1928) demonstrated the effect of moisture in slowing up the oxidative deterioration of stored grain products. I have obtained similar results with stored crackers, and so also have Holm, Wright and Greenbank (1927) in the case of powdered milks. Just how moisture retards rancidity development is not known. Greenbank and Holm (1924) believe that water may extract catalyzing substances from the fat or it may also cause the oxidation to go directly to the acid stage, instead of stopping at the aldehyde stage with the production of a rancid odor and taste.

Metals act as powerful catalysts in the oxidative deterioration of fats, only traces being necessary to effect a vigorous catalysis. This effect of metals is particularly a problem in the spoilage of dairy products, since traces of copper and tin are dissolved from containers in the various stages of processing of milk and milk products. Davies (1928) used the methylene blue reduction test to demonstrate the catalytic effect of metals in increasing the oxidation of butterfat.

High temperatures and light are also catalysts for the oxidation of fats, but of prime importance is the presence of oxygen or air. Storage in the absence of air, as in a vacuum, is a very efficient method of retarding oxidative deterioration in fats. Holm, Greenbank and Deysher (1927) have shown, however, that fat may contain loosely combined oxygen which can effect an oxidation of the fat even in a vacuum when stored over a considerable period of time. Displacement of the air by an inert gas such as nitrogen or hydrogen also prevents the rapid oxidation of fats, while carbon dioxide does not appear to act as an inert gas in this respect and does not materially retard the rate of fat oxidation. Emery and Henley (1922) found lard stored in an atmosphere of carbon dioxide became rancid as

quickly as when stored in air, while Supplee and Dow (1925) found the same to hold true in the storage of milk powder.

Anti-oxygenic Catalysts and the Autoxidation of Fats

Considerable interest has been aroused of late by the possibility of using anti-oxygenic catalysts to prevent or retard the autoxidation of fats. The study of anti-oxygenic catalysts was initiated largely through the results of Moureau and Dufraisse (1926), and has received its greatest impetus by the effective application in preventing the oxidative deterioration of rubber. The action of these substances as inhibitors has been explained by Moureau and Dufraisse on the basis of the theory of "negative catalysis" and by Alyea and Bäckstrom (1929) through the chain reaction theory. These inhibitors apparently function through forming compounds with, or being oxidized by, the peroxides in the fat, thereby decreasing the oxidizing potential of the fat and preventing its rapid oxidation.

In a recent paper Mattill (1931) has investigated the action of a large number of anti-oxidants on fats, and found a number of them (hydroquinone, quinone, pyrocatechol, pyrogallol, α-naphthol and B-naphthoquinone) effective as inhibitors. Mattill attempted to correlate the inhibiting power with the configuration of the inhibitor. His observations indicate that the anti-oxygenic effect is due, in the phenolic compounds, to two hydroxyl groups in the ortho or para positions, while in the naphthols but one hydroxyl group is sufficient.

The fact that substances are known which will retard the autoxidation of fats raises the possibility of their use in the preservation of edible fats. So far, at least, they have been viewed with suspicion due to their questionable toxic effect in foods, and their commercial application has not been attempted so far as we are aware.

It is interesting, however, that an anti-oxygenic substance appears to be normally present in wheat flour. Mattill (1927) found wheat germ oil to exert a protective action in preventing the oxidative destruction of vitamins A and E, and postulated that the sterols present in the oil were responsible for this action. This was later substantiated by Mattill and Crawford (1930).

Triebold (1929) studying the relation of shortening agents to the keeping quality of the crackers found certain cracker samples exhibiting much better keeping qualities than the shortening agents would warrant. He explained this on the basis that the wheat oil, present in the cracker flour, contained some substance which prevented the rapid oxidation of the shortening, an explanation, which is in direct contradiction to the more or less general idea that wheat oil aids in the oxidative deterioration of baked goods.

To test out the assumption that wheat oil acted as an inhibitor in the oxidative deterioration of baked goods, three series of cracker samples were prepared under constant conditions and their keeping qualities determined by the lengths of induction periods of oxygen absorption. One series of samples were baked as controls, another series were baked using the same flour as the controls except that it was first extracted with ether, and a third series used the flour after it had been simply treated with ether and evaporated without the loss of any of the oil.

The results obtained on these three series of cracker samples are given in Table I. It will be noted that the ether extracted flour,

TABLE I

EFFECT OF WHEAT OIL PRESENT IN CRACKED FLOUR ON THE KEEPING QUALITY OF CRACKERS

Sample	Length of induction period in hours. 100 gram samples of crackers and 20 gram samples of shortenings oxidized at 90° C.
Controls	7.0
Ether-treated (not extracted)	7.0
Ether-extracted	3.0
Prime steam lard shortening	4.5

from which the greater percentage of wheat oil had been extracted, showed a relatively short induction period, indicative of poor keeping qualities. Treatment with ether, without extracting the oil, did not affect the keeping qualities of the resulting crackers as they exhibited practically the same length of induction period as the controls. The baked crackers showed a considerably longer induction period than the original shortening, indicating that the keeping qualities of the shortening had been enhanced by the wheat oil in the cracker flour. This study has just been completed and we are continuing along this line at the present time.

Acknowledgment

The writer is indebted to Mr. Charles E. Bode for his aid in the extraction of the flour and the baking of the cracker samples.

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ERRATUM

ON THE SEPARATION OF "GASSING POWER" (DIASTATIC ACTIVITY) FROM "STRENGTH" IN BAKING TESTS

HOLGER JØRGENSEN

On pages 369 and 370 of Vol. VIII, No. 5, of this Journal (September 1931) Figures 2 and 3 are in reverse order. Figure 3 should be labeled Figure 2, and vice versa.

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